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Dendritic cells.

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THANK YOU!

## Dendritic Cells

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## I. Introduction

Dendritic cells (DCs) were first identified in the epidermis in 1868, and were termed Langerhans cells (Langerhans, 1868). Their presence in other tissues was identified a century later in 1973 (Steinman and Cohn, 1973). DCs are now recognized as an integral part of the lymphohematopoietic system, and function as sentinels of the immune system, initiating immune responses. DCs are found in the interstitium of most organs (excluding brain) at a frequency so low that this has posed a major impediment to their study. The cells can usually be identified by their characteristic and unusual morphology, as well as their high-level expression of class II MHC molecules.

To launch immune responses, DCs have to capture small amounts of antigen efficiently and present it to rare antigen-specific T cells to initiate their expansion and maturation (Fig. 1). These two key functions of DCs segregate in time and space. The soluble or particulate antigen/pathogen that invades tissues is efficiently captured by tissue DCs. This triggers DC migration into the proximal secondary lymphoid organ, where they mature into a developmental state that allows the selection and activation of antigen-specific T cells. In particular, DCs support the generation of not only lymphokine-secreting helper T cells, but also effector cytotoxic T lymphocytes (CTLs), which subsequently migrate to the site of initial injury to eliminate virally infected cells or tumor cells. This capacity of activating not only memory, but also naive T cells, is a property not shared by other antigen-presenting cells (APCs). Hence, DCs are in fact professional APCs.

Knowledge of DC physiology has progressed considerably because of the discovery of culture techniques, in the early 1990s, that support the *in vitro* generation of large numbers of DCs from hematopoietic progenitors. DCs comprise three distinct subsets, including two within the myeloid lineage, Langerhans cells and interstitial DCs, and one within the lymphoid lineage, the so-called lymphoid DC subset. There are three stages of development, i.e., precursor DCs (DC<sub>pre</sub>) patrolling through blood and lymphatics, immature DCs (DC<sub>imm</sub>) residing within virtually every tissue

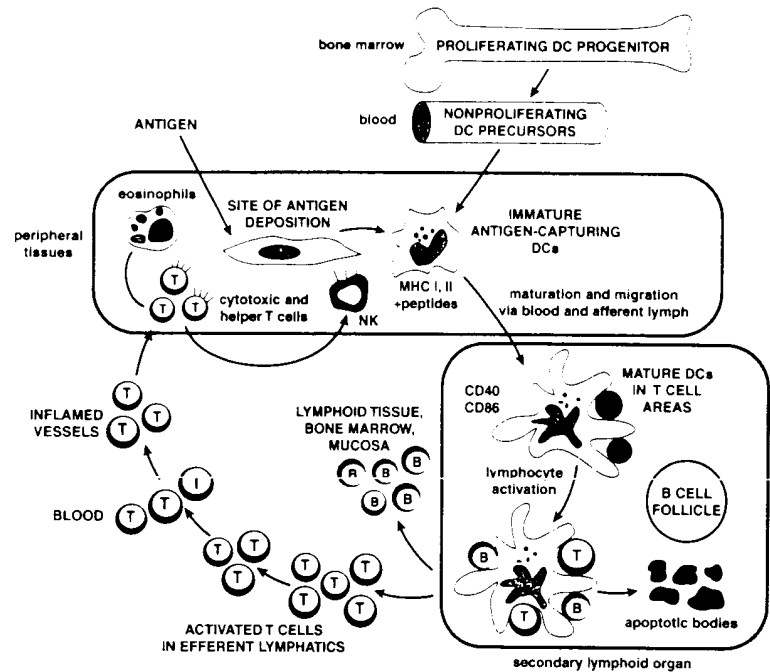
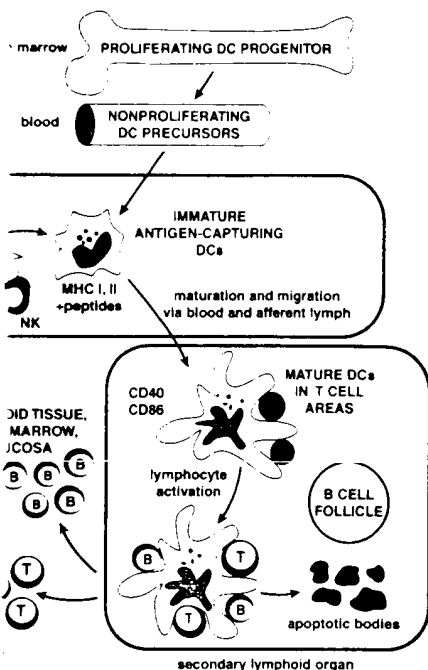


FIG. 1. *The life of a dendritic cell (DC) or the capture of antigens and their presentation to selected antigen-specific lymphocytes.* Circulating precursor DCs enter peripheral tissues as immature DCs, where they are poised to capture antigens (e.g., microbial products). Loaded immature DCs leave the tissues and migrate to lymphoid organs, where, after maturation, they display antigen-derived peptides on their MHC molecules, which, in turn select rare circulating antigen-specific lymphocytes. These reactive T cells become activated and further induce terminal DC maturation, which supports lymphocyte expansion and differentiation. Activated T lymphocytes migrate back to the injured tissue, because they can selectively traverse inflamed epithelium. Helper T cells secrete lymphokines, and cytotoxic T cells eventually lyse the infected cells. Activated B cells differentiate into B lymphoblasts after contact with T cells and DCs, and then migrate into various areas, where they mature into plasma cells and produce antibodies that will eventually neutralize the initial pathogen.

in ambush to capture pathogens, and mature DCs ( $DC_{mat}$ ) residing temporarily within secondary lymphoid organs. In addition to being involved in the initiation of immunity, DCs also appear to play an important role in the induction of immunological tolerance. In particular, thymic DCs present endogenous self-peptides to newly generated thymocytes, thereby allowing the deletion of self-reactive T cells. These thymic DCs may indeed originate from a precursor cell that also gives rise to lymphocytes and natural killer



of the capture of antigens and their presentation. Circulating precursor DCs enter peripheral tissues to capture antigens (e.g., microbial products), and migrate to lymphoid organs, where, after presentation of antigens on their MHC molecules, which, in turn, activate T cells. These reactive T cells become activated T cells, which supports lymphocyte expansion and migration back to the injured tissue, because they can secrete lymphokines, and cytotoxic T cells. Activated B cells differentiate into B lymphoblasts when they migrate into various areas, where they mature and eventually neutralize the initial pathogen.

and mature DCs (DC<sub>mat</sub>) residing temporarily in lymphoid organs. In addition to being involved in the immune response, they also appear to play an important role in the immune response. In particular, thymic DCs present self-generated thymocytes, thereby allowing self-censorship. These thymic DCs may indeed originate from hematopoietic progenitors that give rise to lymphocytes and natural killer

(NK) cells, and have thus been called lymphoid DCs. There is also evidence of a role for DCs in the development of peripheral tolerance. Recent studies further indicate that DCs can directly modulate B cell and NK cell functions. Molecular genetic approaches are also ascribing to DCs new molecules, such as chemokines and chemokine receptors, proteases, and antiproteases, lectinlike receptors for antigen uptake, new members of the TNF/TNF receptor family, as well as killer inhibitory receptors. It is hoped that this will increase understanding of the biological functions of DCs, selectively identify immature and mature DCs, and explain DC development at the signaling and transcriptional levels. DC research is further fueled by the hope that cultured DCs will lead to the development of cellular vaccines for use in cancer therapy and the treatment of various infectious diseases.

## ii. Features of Dendritic Cells

### A. MORPHOLOGY

Figure 2 (see color plate) illustrates the unusual shape that gives rise to the term "dendritic cell." *In situ*, as in the skin and lymphoid organs, immature and mature DCs have a stellate shape. Many fine dendrites are displayed when DCs are isolated and spun onto slides; DCs extend large sheetlike processes or veils in many directions from the cell body. The processes are long (10  $\mu$ m) and thin, either fine or sheetlike. Actin cables are scarce (Winzler *et al.*, 1997). The shape and motility of DCs suit their functions, initially the efficient capture of antigen and subsequently the selection of rare antigen-specific lymphocytes.

### B. PRECURSOR DENDRITIC CELLS, IMMATURE DENDRITIC CELLS, AND MATURE DENDRITIC CELLS

All tissues, with the possible exception of brain and testis, contain DCs that are immature (DC<sub>imm</sub>), capable of capturing antigens but not yet possessing the panel of accessory molecules required for potent T cell stimulation. Antigens able to drive an immune response are those that efficiently initiate the maturation of DCs. *In vivo*, transplantation (Larsen *et al.*, 1990a,b; 1994) and contact allergens (Enk *et al.*, 1993a,b; Silberberg-Sinakin *et al.*, 1976) are among the most powerful immunologic stimuli for DC maturation.

The best studied DC<sub>imm</sub> is certainly the epidermal Langerhans cell (LC), which was shown to be derived from hematopoietic progenitors using bone marrow reconstitution experiments (Katz *et al.*, 1979). LCs, identified by expression of the CD1a antigen (Fig. 3) and the presence of Birbeck granules (cytoplasmic structures formed by double membrane joinings),

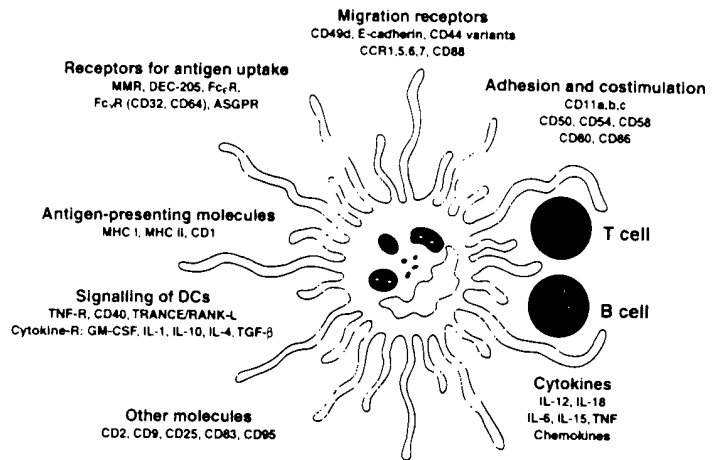
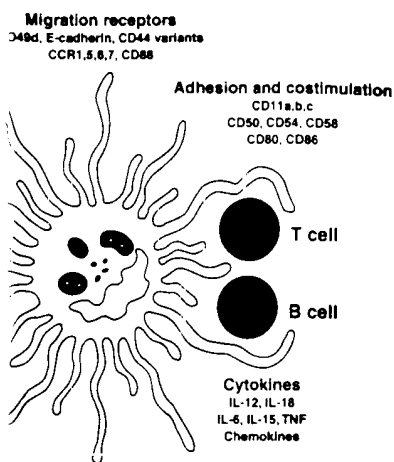


FIG. 3. *Molecules expressed by dendritic cells.* Illustrated are the key features used in combination to identify DCs. At the present time there is not a single molecule that permits unambiguous assignment of a given cell to the DC family. The combination of several markers, however, defines a dendritic cell subpopulation and its stage of maturation.

are localized to the basal and suprabasal layers of the epidermis (Katz *et al.*, 1979; Romani *et al.*, 1985). The CD1 antigens are nonpolymorphic cell surface proteins noncovalently associated with  $\beta_2$ -microglobulin and bear some structural similarity to major histocompatibility complex (MHC) molecules. CD1 molecules have been shown to present peptides as well as microbial, nonpeptide, lipid-containing antigens to T cells (Maher and Kronenberg, 1997; Porcelli *et al.*, 1992). The CD1a antigen is also a cortical thymocyte marker that disappears at later stages of T cell maturation (McMichael *et al.*, 1979). Other members of the CD1 family have also been identified on LCs. LCs express variable amounts of CD1c (Davis *et al.*, 1988), and higher percentages of CD1b<sup>+</sup> cells are present among dermal and migrating LCs (Richters *et al.*, 1996).

Interstitial DCs in most organs and tissues, such as lung (Gong *et al.*, 1992; Havenith *et al.*, 1993; Holt, 1993; Schon-Hegrad *et al.*, 1991; Xia *et al.*, 1995), heart and kidney (Austyn *et al.*, 1994), and dermis (Nestle *et al.*, 1998b, 1993), represent an important reservoir of DC<sub>imm</sub>. These cells differ from Langerhans cells in that they lack Birbeck granules and do not always express CD1 antigens. After antigen exposure or inflammatory stimuli, DC<sub>imm</sub> migrate via afferent lymph as "veiled DCs," to the draining lymph nodes where they localize to the T cell areas as mature interdigitating



tic cells. Illustrated are the key features used in at time there is not a single molecule that permits to the DC family. The combination of several subpopulation and its stage of maturation.

erabasal layers of the epidermis (Katz *et al.*, 1993; Schon-Hegrad *et al.*, 1991; Xia *et al.*, 1994), and dermis (Nestle *et al.*, 1994). These cells are an important reservoir of DC<sub>imm</sub>. These cells are characterized by the fact that they lack Birbeck granules and do not contain antigen. After antigen exposure or inflammatory stimuli, they migrate to lymph as "veiled DCs," to the draining lymph node as mature interdigitating cells (IDCs).

DCs (IDCs). IDCs are also present in other secondary lymphoid organs such as tonsils and the white pulp of spleen (Bjorck *et al.*, 1997b; Hart and McKenzie, 1988; Steinman, 1991).

Before the availability of DC cultures, LCs provided the most suitable experimental model for studying maturation. Freshly isolated LCs express antigens and detectable Fcγ receptors (CD32/FcγRII and CD64/FcγRI), as well as high-affinity IgE receptors (FcεRI) that contribute to antigen capture. However, LCs are not particularly potent APCs for the mixed lymphocyte reaction (MLR). In contrast, LCs maintained in culture for several days resemble DC<sub>mat</sub> in phenotype and function, including their capacity to initiate T cell responses to alloantigen in the MLR (Romani *et al.*, 1989; Schuler and Steinman, 1985; Teunissen *et al.*, 1990).

During maturation DCs undergo major changes in phenotype and function (Fig. 4). The new phenotype distinguishes DC<sub>mat</sub> from DC<sub>imm</sub> based on critical epitopes such as CD83, CD80, and CD86. The CD83 antigen is a 186-aa single-chain glycoprotein, member of the immunoglobulin superfamily) is presently one of the most useful markers for identification of DC<sub>mat</sub> (Zhou *et al.*, 1992; Zhou and Tedder, 1995b). CD83<sup>+</sup> cells express the highest levels of MHC class II molecules, when compared with other leukocyte lineages, and immunohistologic analysis reveals that CD83 is found mainly on DCs within T lymphocyte areas of lymphoid organs. A DC

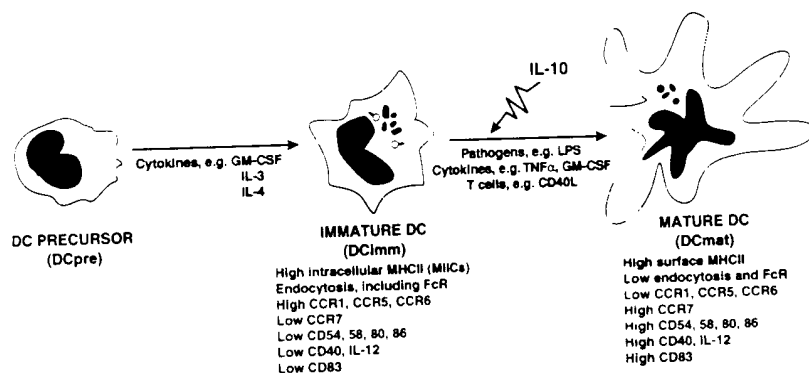


FIG. 4. Stages of dendritic cell maturation. DC precursors, which originate from CD34<sup>+</sup> bone marrow progenitors, circulate in the blood as nonlymphoid mononuclear cells or monocytes, identifiable as a class II MHC-positive CD11c<sup>+</sup> DC<sub>pre</sub> or CD11c<sup>+</sup> DC<sub>pre</sub>. These precursors migrate into tissues to become resident DC<sub>imm</sub>, and this may increase in response to inflammatory cytokines. After antigen capture, DC<sub>imm</sub> undergo maturation during migration to secondary lymphoid organs. Maturation is completed after the selection, activation, and interaction with antigen-specific T cells. In simple terms, maturation transforms an antigen-capturing cell into an antigen-presenting, lymphocyte-activating cell.

that is a novel member of the lysosome-associated membrane glycoprotein (LAMP) family, homologous to the lysosomal marker CD68, has been cloned by screening a cDNA library of *in vitro*-generated DCs. It is not expressed on interstitial DCs but is uniquely expressed by DC<sub>mat</sub> as shown by specific staining of interdigitating DCs within secondary lymphoid organs (De Saint Vis *et al.*, 1998). The antigen recognized by the CMRF44 monoclonal antibody, most likely a glycolipid, is expressed at high density on mature DCs and its expression increases very quickly on blood DC<sub>pre</sub> cultured *in vitro* (Fearney *et al.*, 1997; Hock *et al.*, 1994). During maturation, several other molecules are up-regulated, including class II MHC antigens (Fearney *et al.*, 1997; Hock *et al.*, 1994; Said *et al.*, 1997; Schuler and Steinman, 1985; Witmer-Pack *et al.*, 1985), ICAM-1 (CD54), LFA-3 (CD58), CD11a/c, CD40, CD80 (Inaba and Steinman, 1984; Larsen *et al.*, 1992; Lenz *et al.*, 1993; Young *et al.*, 1992), and CD86 (Caux *et al.*, 1994c; Inaba *et al.*, 1995). The actin-bundling protein p55 fascin, a molecule involved in the organization of the actin cytoskeleton that supports the formation of dendritic processes (Mosialos *et al.*, 1996; Ross *et al.*, 1998), also increases with differentiation. In contrast, Fc receptor expression decreases substantially during DC maturation. Some chemokine receptors are also down-regulated, whereas others are up-regulated, thus supporting the appropriate homing of DCs at their various stages of differentiation (Dien *et al.*, 1998; Sozzani *et al.*, 1998).

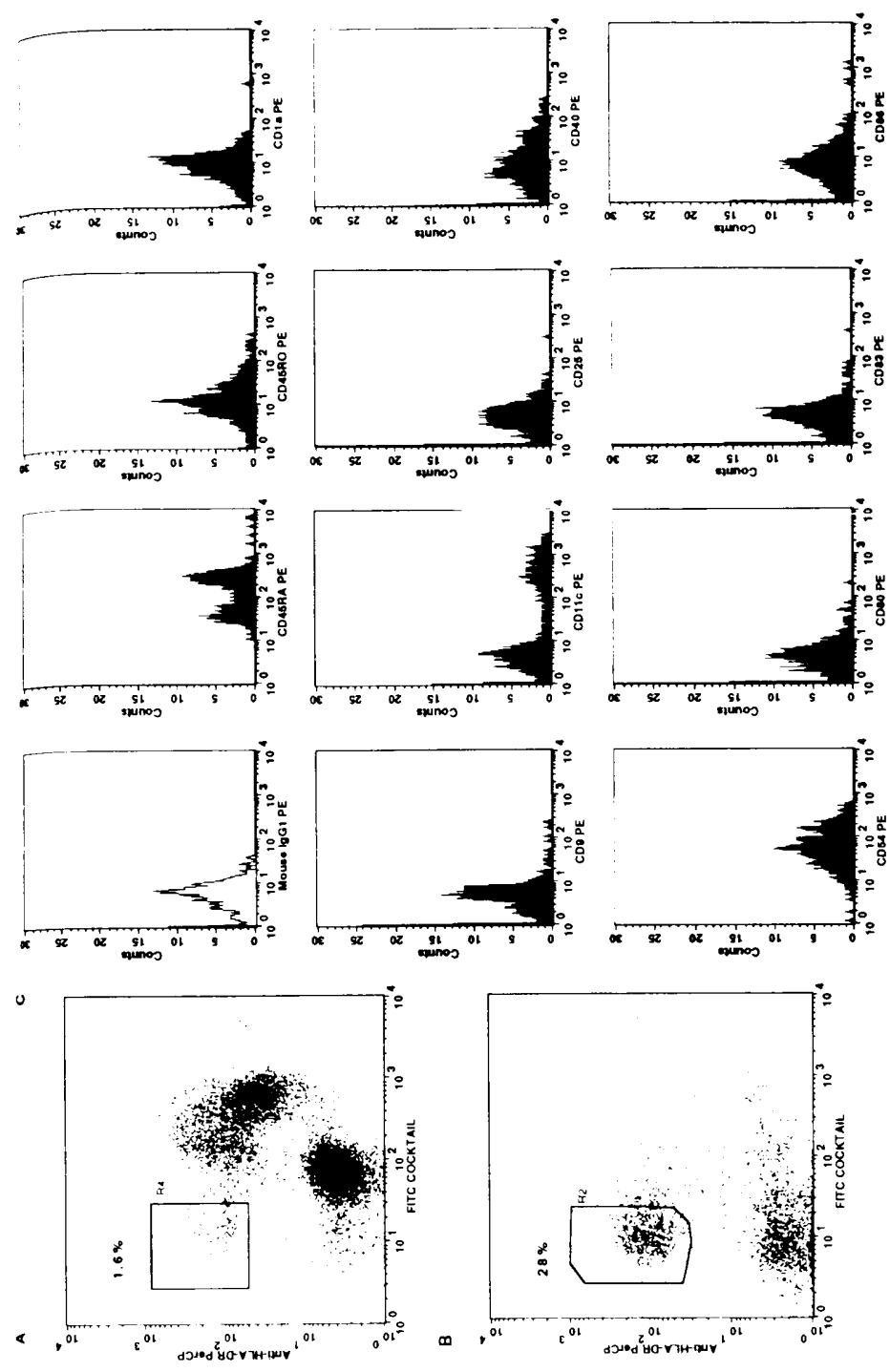
Within blood and lymphoid organs, two populations of cells with some characteristics of DCs have been identified (Fig. 5). These cells are distinguished from other lymphoid and myeloid cells by their high levels of class II MHC and lack of CD3, CD19, CD14, and CD56. One population, CD4<sup>+</sup>, CD11c<sup>+</sup>, CD13<sup>+</sup>, and CD33<sup>+</sup>, mostly found within germinal centers but also in the circulation, displays a morphology of immature DCs and quickly matures *in vitro* (Grouard *et al.*, 1996; O'Doherty *et al.*, 1993; Thomas *et al.*, 1993). The other population, CD4<sup>+</sup>, CD11c<sup>+</sup>, CD13<sup>+</sup>, CD33<sup>+</sup>, and CD123<sup>+</sup>, resembles the morphology of plasma cells and corresponds to the enigmatic plasmacytoid T cells that are restricted to the T cell-rich areas of secondary lymphoid organs (Grouard *et al.*, 1997; Olweus *et al.*, 1997). Interestingly, this population undergoes very rapid apoptosis in culture unless rescued by IL-3. These CD11c<sup>+</sup> CD123<sup>+</sup> cells differentiate into cells with DC characteristics in response to IL-3 and CD40L.

FIG. 5. CD11c<sup>+</sup> and CD11c<sup>+</sup> dendritic cell precursors from the peripheral blood. Blood mononuclear cells display ~1% HLA-DR<sup>high</sup> lineage<sup>+</sup> CD3<sup>+</sup>, CD14<sup>+</sup>, CD16<sup>+</sup>, CD19<sup>+</sup>, CD56<sup>+</sup> cells (A), which can be enriched to as high as 30% after bead depletion (B). A third fluorochrome (C) identifies additional epitopes expressed by CD11c<sup>+</sup> and CD11c<sup>+</sup> DC<sub>pre</sub>.

some-associated membrane glycoprotein the lysosomal marker CD68, has been a part of *in vitro*-generated DCs. It is not uniquely expressed by DC<sub>mat</sub> as shown by DCs within secondary lymphoid organs. The antigen recognized by the CMRF44 antibody, a glycolipid, is expressed at high density on DCs and increases very quickly on blood DC<sub>mat</sub> (Hock *et al.*, 1994; Said *et al.*, 1997; Schuler *et al.*, 1997). During maturation, DCs up-regulate, including class II MHC (Hock *et al.*, 1994; Said *et al.*, 1997; Schuler *et al.*, 1997), ICAM-1 (CD54), LFA-3 (CD58) (Inaba and Steinman, 1984; Larsen *et al.*, 1992), and CD86 (Caux *et al.*, 1992), the actin-binding protein p55, fascin, a molecule of the actin cytoskeleton that supports cell motility (Mosialos *et al.*, 1996; Ross *et al.*, 1996). In contrast, Fc receptor expression decreases during DC maturation. Some chemokine receptors are up-regulated, thus regulating DCs at their various stages of differentiation (Inaba *et al.*, 1998).

Thus, two populations of cells with some overlap were identified (Fig. 5). These cells are distinct from myeloid cells by their high levels of class II MHC, CD14, and CD56. One population, CD11c<sup>+</sup>, mostly found within germinal centers, has a morphology of immature DCs and is distinct from CD11c<sup>+</sup> DCs (O'Doherty *et al.*, 1992). The other population, CD4<sup>+</sup>, CD11c<sup>+</sup>, CD13<sup>+</sup>, has the morphology of plasma cells and corresponds to T cells that are restricted to the T cell zone (Grouard *et al.*, 1997; Olweus *et al.*, 1997). This population undergoes very rapid apoptosis. These CD11c<sup>+</sup> CD123<sup>+</sup> cells differentiate into DCs in response to IL-3 and CD40L.

**Fig. 5.** DC cell precursors from the peripheral blood. Blood from a patient with a high level of DCs (CD3<sup>+</sup>, CD14<sup>+</sup>, CD16<sup>+</sup>, CD19<sup>+</sup>, CD56<sup>+</sup>) was isolated. A: A third population of DCs was identified by flow cytometry. B: A third population of DCs was identified by flow cytometry.



These DCs lack the myeloid cell markers CD13 and CD33 and may thus be the human lymphoid DCs. Importantly, these mature DCs appear to induce naive T cells to differentiate specifically along the Th2 pathway, as demonstrated by the secretion of IL-4 and the lack of IFN- $\gamma$  (Y. J. Liu, personal communication). A working hypothesis is that these two cell populations correspond to patrolling precursor DCs (DC<sub>pre</sub>) that home to sites of injury, from which DC<sub>imm</sub> have earlier fled in their migration toward draining lymphoid organs. This influx of DC<sub>pre</sub> can be measured within 30 min, whereas accumulation of neutrophils requires 4 hr (McWilliam *et al.*, 1996).

### C. PHENOTYPE

Figure 3 illustrates important molecules that distinguish subpopulations and stages of maturation of dendritic cells.

#### 1. Antigen Capture and Presentation by Immature DCs

*a. Antigen Capture.* DC<sub>imm</sub> can efficiently internalize a diverse array of antigens for processing and loading onto class II MHC molecules, as a consequence of high endocytic activity levels. Antigen uptake by DC<sub>imm</sub> can occur via four distinct mechanisms: (1) macropinocytosis, (2) receptor-mediated endocytosis through Fc $\gamma$  and Fc $\epsilon$  receptors (Maurer *et al.*, 1996; Sallusto and Lanzavecchia, 1994), (3) receptor-mediated endocytosis through the mannose receptor (Sallusto *et al.*, 1995) and C-type lectin receptor DEC205 (Jiang *et al.*, 1995), and (4) engulfment of apoptotic bodies through the vitronectin receptor  $\alpha(v)\beta 3$  (Albert *et al.*, 1998; Rubartelli *et al.*, 1997).

*1. Macropinocytosis.* Macropinocytosis is a cytoskeleton-dependent type of fluid-phase endocytosis mediated by membrane ruffling and the formation of large vesicles (1–3  $\mu$ m). In DCs, macropinocytosis is constitutive, and enables a single cell to take up a very large volume of fluid (half the cell's volume per hour) (Sallusto *et al.*, 1995).

*2. Fc $\epsilon$  and Fc $\gamma$  receptors.* Human epidermal LCs, but not other epidermal cells, express Fc $\epsilon$ RI (Kraft *et al.*, 1998; Rieger *et al.*, 1992; Wang *et al.*, 1992) and use this receptor to maximize antigen uptake via specific IgE for subsequent presentation to T cells (Bieber, 1997). The Fc $\epsilon$ RI on DCs is a multimeric receptor composed of the  $\alpha$  and  $\gamma$  chains initially identified on basophils, but lacking the  $\beta$  chain (Maurer *et al.*, 1996). LCs also express the low-affinity Fc $\epsilon$ RII, CD23, which may have a role in the pathogenesis of atopic eczema as well as in the regulation of IgE synthesis (Bieber *et al.*, 1989). In response to maturation stimuli, immature DCs down-regulate their Fc receptors for IgG, Fc $\gamma$ RI (CD64) (Fanger *et al.*, 1996), and Fc $\gamma$ RII (CD32) (Thomas *et al.*, 1993), thereby reducing their antigen capture by this mechanism.

CD13 and CD33 and may thus be these mature DCs appear to induce along the Th2 pathway, as demonstrated by the lack of IFN- $\gamma$  (Y. J. Liu, personal communication). It is that these two cell populations (DC<sub>pre</sub>) that home to sites of injury, their migration toward draining lymph nodes can be measured within 30 min, and their numbers increase within 4 hr (McWilliam *et al.*, 1996).

These data distinguish subpopulations of DCs.

#### Antigen Uptake by Immature DCs

Immature DCs efficiently internalize a diverse array of antigens into class II MHC molecules, as a result of several mechanisms at the cellular level. Antigen uptake by DC<sub>imm</sub> involves (1) macropinocytosis, (2) receptor-mediated endocytosis, (3) Fc $\epsilon$ R1 receptors (Maurer *et al.*, 1996; Moll *et al.*, 1997), (4) receptor-mediated endocytosis of IgG (Moll *et al.*, 1995) and C-type lectin receptors (Moll *et al.*, 1995) and (5) engulphment of apoptotic cells (Albert *et al.*, 1998; Rubartelli *et al.*, 1997).

Macropinocytosis is a cytoskeleton-dependent process mediated by membrane ruffling and the formation of macropinosomes. In DCs, macropinocytosis is constitutive and involves the uptake of a very large volume of fluid (half the volume of the cell) (Moll *et al.*, 1995).

In epidermal LCs, but not other epidermal cells (Moll *et al.*, 1998; Rieger *et al.*, 1992; Wang *et al.*, 1992), macropinocytosis is the primary mechanism for antigen uptake via specific receptors (Bieber, 1997). The Fc $\epsilon$ R1 on the surface of the  $\alpha$  and  $\gamma$  chains initially binds to the antigen-antibody complex (Maurer *et al.*, 1996). LCs express CD23, which may have a role in the regulation of IgE synthesis. In the presence of maturation stimuli, immature DCs express Fc $\gamma$ R1 (CD64) (Fanger *et al.*, 1993), thereby reducing their

3. *The mannose receptor and C-type lectin receptor DEC-205.* DCs express high levels of the mannose receptor, which contains multiple carbohydrate-binding domains and is involved in the internalization of a variety of glycoproteins. Whereas Fc receptors are degraded together with their cargo, the mannose receptor releases its ligand at endosomal pH and is recycled. This allows uptake and accumulation of many ligands by a small number of receptors (Engering *et al.*, 1997; Lanzavecchia, 1996). The mannose receptor may play a critical role in phagocytosis of particles and microbes (Inaba *et al.*, 1983b; Moll *et al.*, 1993; Reis e Sousa *et al.*, 1993; Svensson *et al.*, 1997). Another endocytic receptor is DEC-205, an integral membrane protein homologous to the mannose receptor. DEC-205 and its antigenic ligand are rapidly taken up by means of coated pits and vesicles, then delivered to a multivesicular endosomal compartment that resembles the class II MHC-containing vesicles implicated in antigen presentation (Geuze, 1998a; Jiang *et al.*, 1995).

4. *Engulphment of apoptotic bodies.* DCs are able, *in vitro* and *in vivo*, to capture and engulf apoptotic cells (Albert *et al.*, 1998; Rubartelli *et al.*, 1997). Immature DCs appear to be more efficient than mature DCs in capturing apoptotic bodies as a means of antigen uptake (M. L. Albert and N. Bhardwaj, personal communication). Although macrophages engulf apoptotic bodies using multiple surface molecules (CD14, CD36, phosphatidylserine receptor) (Devitt *et al.*, 1998; Rubartelli *et al.*, 1997), DCs may preferentially use the vitronectin receptor  $\alpha v\beta 3$  and the CD36/thrombospondin receptor (J. Banchereau, unpublished observations). The engulphment of apoptotic bodies induces a rise in intracellular free calcium concentration [ $Ca^{2+}$ ]<sub>i</sub>, which is essential for the engulphment to occur (Rubartelli *et al.*, 1997). Apoptosis, but not necrosis, is required for the generation and packaging of immunogenic material for delivery to DCs. In particular, DCs loaded with apoptotic bodies, derived from either macrophages or HeLa cells infected with influenza virus, can stimulate the proliferation of influenza specific T cells and the generation of class I MHC-restricted, influenza-specific CD8<sup>+</sup> CTLs (Albert *et al.*, 1998; Huang *et al.*, 1994). This pathway is likely to account for the *in vivo* phenomenon of "cross-priming" (Bevan, 1977), whereby antigens derived from tumor cells (Inaba *et al.*, 1998) or transplants (Fossum and Rolstad, 1986) are presented by host APCs. Tolerance to tissue-restricted self antigens may also depend on apoptotic cell death, as occurs during development and normal cell turnover. The specifics are not established, but this could be followed by antigen presentation by DCs (Kurts *et al.*, 1996, 1997b), with a resultant nonproliferative or anergic T cell response. Interestingly, while macrophages can also engulf apoptotic bodies, they are unable to stimulate

specific CTLs. Furthermore, they even prevent DC-mediated CTL generation by this route by sequestering antigen (Albert *et al.*, 1998).

*b. Antigen Presentation—MHC Class II and MHC Class I Molecules.*

*1. MHC class II loading.* MHC class II loading is critical for CD4 T cells. In addition to efficient antigen capture, DCs fulfill other requirements for antigen presentation by synthesizing and expressing high levels of class II MHC (Kleijmeer *et al.*, 1994, 1995; Young *et al.*, 1992). Considerable evidence indicates that late endosomes (which develop from the vacuolar parts of the early endosomes network) and their lysosomal derivatives play a crucial role in class II MHC-mediated antigen presentation (Geuze, 1998b; Pierre and Mellman, 1998). In APCs, and most particularly DCs, the majority of intracellular class II MHC is found in late endocytic structures with numerous internal membrane vesicles and sheets, collectively designated MIICs (MHC class II compartments). A minor compartment is represented by early endosomes that contain mature class II MHC molecules, which are internalized from the cell surface and rapidly recycled (Harding and Unanue, 1989; Reid and Watts, 1990). The major compartment (MIIC) contains newly synthesized class II MHC molecules that are targeted to this structure by the invariant chain (Ii). It also contains HLA-DM molecules that remove the Ii-derived class II-associated invariant chain peptide (CLIP) and promote the formation of stable complexes (Lanzavecchia, 1996). Cell fractionation studies have indicated the presence of class II MHC-positive vesicles (CIIV) that are physically and biochemically distinct from conventional endosomes and lysosomes (Pierre *et al.*, 1997). During DC maturation, three sequential stages are identified: early DCs, in which class II MHC antigens are localized to lysosomal compartments; intermediate DCs that accumulate class II in distinctive nonlysosomal vesicles; and mature DCs, in which peptide-class II MHC complexes are present on the plasma membrane for long periods of time, thereby allowing the selection of rare antigen-specific T cells (Cella *et al.*, 1997c; Pierre *et al.*, 1997c).

*2. MHC class I loading.* MHC class I loading is critical for CD8 T cells. Professional APCs can capture exogenous antigens for presentation on MHC class I molecules. This ensures an efficient generation of cytotoxic CD8<sup>+</sup> T cells (Heemels and Ploegh, 1995; Watts, 1997), even against viral or tumor antigens that are expressed only in nonprofessional APCs. *In vitro* experiments suggest two fundamentally different pathways for the presentation of exogenous antigens: (1) one involving unconventional post-Golgi loading of MHC class I (Harding and Song, 1994; Liu *et al.*, 1995) and (2) another one involving the classical transporter associated with antigen processing (TAP) loading mechanism (Rock *et al.*, 1986). *In vitro*

revent DC-mediated CTL generation (Albert *et al.*, 1998).

**Class II and MHC Class I Molecules.** Class II loading is critical for CD4 T cells, and DCs fulfill other requirements and expressing high levels of class II (Young *et al.*, 1992). Considerable evidence indicates that late endosomes and their lysosomal derivatives played a role in antigen presentation (Geuze, 1997). APCs, and most particularly DCs, contain class II molecules that are found in late endocytic structures (multivesicular bodies and sheets, collectively termed late endosomes). A minor compartment that contains mature class II MHC molecules on the cell surface and rapidly recycled (Watts, 1990). The major compartment contains class II MHC molecules that are associated with invariant chain (Ii). It also contains HLA-DM, which is involved in the formation of stable complexes between class II and peptide. These studies have indicated the presence of class II MHC molecules in late endosomes and lysosomes (Pierre *et al.*, 1997). The sequential stages are identified: class II molecules are localized to lysosomes and accumulate class II in distinctive compartments in which peptide-class II MHC complexes are formed for long periods of time, and then presented to T cells (Cella *et al.*, 1997).

Class I loading is critical for CD8 T cells, and DCs are efficient for presentation of exogenous antigens for presentation (Watts, 1997), even against viral antigens in nonprofessional APCs. In contrast to different pathways for the presentation of exogenous antigens involving unconventional post-translational modification and Song, 1994; Liu *et al.*, 1995) and a specific transporter associated with antigen presentation (Rock *et al.*, 1986). *In vitro*

cross-priming requires a functional TAP pathway (Huang *et al.*, 1994; Norbury *et al.*, 1997). The peptides for class I MHC on DCs can be derived from nonreplicating microbes (Svensson *et al.*, 1997), soluble proteins (Norbury *et al.*, 1997), or apoptotic cells (Albert *et al.*, 1998).

Experiments using a unique class I MHC<sup>+</sup>/class II MHC<sup>-</sup>/CD80<sup>+</sup> dendritic cell line (80/1DC) derived from murine fetal skin have led to the conclusion that direct allogeneic class I MHC-restricted immunity can occur in the absence of class II expression (Kolesaric *et al.*, 1997; Lenz *et al.*, 1996). This mechanism has biological relevance to transplantation immunity, as well as immunity against opportunistic infections in conditions of congenital, iatrogenic, or acquired immunodeficiencies.

## 2. Adhesion Molecules

During their migration and subsequent interaction with T cells, DCs are involved in a variety of adhesion events. Expression of cutaneous lymphocyte antigen (CLA) may allow DCs to reach the skin by interacting with E-selectin (CD62E) on activated endothelial cells (Strunk *et al.*, 1996; 1997). LCs adhere to the surrounding keratinocytes through homotypic interactions involving E-cadherin. After antigen capture, LCs down-regulate E-cadherin, losing adhesive interactions with surrounding keratinocytes and allowing migration from the skin (Tang *et al.*, 1993). Interestingly, following epicutaneous stimulation with haptens, LCs produce type IV collagenase (MMP 9), which probably facilitates the crossing of the basement membrane (Kobayashi, 1997). Integrins and intercellular adhesion molecules contribute to DC adhesion and migration through vessel walls (Jakob *et al.*, 1997). Immature blood DCs can enter the lymphoid organs through high endothelial venules via CD49d  $\beta$ -integrin (Brown *et al.*, 1997). ICAM-1, which together with ICAM-2 is up-regulated on DC activation and may contribute to DC migration as well as to the later phases of T lymphocyte activation. ICAM-3, the predominant LFA-1 ligand on resting blood DCs, is probably used for initial DC-T cell interactions (Hart and Prickett, 1993; Starling *et al.*, 1995).

## 3. Migration of Dendritic Cells

**a. Patterns of Dendritic Cell Migration.** An important attribute of DCs at various stages of their maturation is their mobility. This property enables DCs to move from the blood to peripheral tissues, and from peripheral tissues to lymphoid organs, where the pool of quiescent T cells recirculates. The selective migration of DCs, their residence in a given tissue, and their migratory capacity are tightly regulated events.

The induced migration of DCs was first noted at the site of contact allergy (Lens *et al.*, 1983; Silberberg-Sinakin *et al.*, 1976). Transplantation

of heart or skin is also accompanied by an efflux of DCs from the graft (Larsen *et al.*, 1990a,b, 1994). In normal lung tissue, a functionally and morphologically identical DC population exists within the epithelial lining of the conductive airways of both humans and rodents, forming a contiguous network analogous to the LC population in the epidermis (Lipscomb *et al.*, 1995). Brief exposure to aerosolized bacterial endotoxin induces a transient increase (~50%) in the density of airway epithelial DCs for 24–48 hr after exposure, suggesting active participation by DCs in the acute inflammatory response (Schon-Hegrad *et al.*, 1991). Within the respiratory tract, inhalation of bacteria, viruses, or soluble protein antigens (McWilliam *et al.*, 1996) rapidly recruits DC<sub>pre</sub> into the airway epithelium. The earliest detectable cellular response after inhalation of *Moraxella catarrhalis* is the recruitment of putative class II MHC-bearing DC<sub>pre</sub> into the airway epithelium, the initial wave arriving earlier than neutrophil influx. Unlike neutrophils, which rapidly transit through the epithelium and into the airway lumen, the DC<sub>pre</sub> remain within the epithelium during the acute inflammatory response. Here they differentiate and develop the dendritic morphology typical of resident DCs found in normal epithelium (McWilliam *et al.*, 1994), subsequently migrating to the regional lymph nodes. In the intestinal lumen antigens are taken up by specialized epithelial cells (M cells) overlying the dome region of Peyer's patches. Immature DCs, strategically located below the M cells, capture the incoming antigens (Ruedl *et al.*, 1996) and migrate to the T cell areas of the same Peyer's patches or draining mesenteric lymph nodes, where they present antigen to T cells (Kelsall and Strober, 1996). After intravenous injection of inert particles, particle-laden cells can be detected in the hepatic lymph (Kudo *et al.*, 1997; Matsuno *et al.*, 1996). These cells may represent DC<sub>pre</sub>, recently derived from monocytes, and recruited to the hepatic sinusoids by phagocytosing Kupffer cells. These DC<sub>pre</sub> manifest temporary phagocytic activity for intravascular particles, which is in turn down-regulated on maturation and translocation from the sinusoidal area to the hepatic lymph (Cella *et al.*, 1997c).

*b. Control of Dendritic Cell Migration.* Although the pathways of DC migration are relatively well characterized, the molecular mechanisms that control recruitment and migration of DCs are far less well defined. Chemotactic factors released by the target tissue and surface adhesins are involved in these processes (Girolomoni and Ricciardi-Castagnoli, 1997). Several approaches have demonstrated that IL-1 and TNF- $\alpha$  are involved in the activation and mobilization of Langerhans cells (LCs). In particular, contact allergens that induce emigration of Langerhans cells induce an accumulation of IL-1 and TNF within the epidermis (Enk *et al.*, 1993a,b), and

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antibodies to IL-1 and TNF inhibit contact allergen-induced sensitization  
and LC redistribution (Cumberbatch and Kimber, 1995). More directly,  
dermal injection of IL-1 or TNF induces a decrease in LCs within the  
epidermis, together with an increase in DCs in the draining lymph nodes  
(Cumberbatch *et al.*, 1992, 1994, 1997; Cumberbatch and Kimber, 1992).  
These cytokines act by down-regulating the surface expression of E-  
cadherin on LCs, thereby loosening their interactions with keratinocytes  
(Blauvelt *et al.*, 1995; Jakob and Udey, 1998; Schwarzenberger and Udey,  
1996; Tang *et al.*, 1993). DCs likewise migrate from the kidney and heart  
in response to IL-1 and TNF (Roake *et al.*, 1995).

DCs can both produce and respond to chemokines, e.g., IL-8 (Zhou  
and Tedder, 1995a), MIP-1 $\alpha$  and MIP-1 $\beta$ , RANTES (Sozzani *et al.*, 1995),  
and MIP-1 $\gamma$  (Mohamadzaheh *et al.*, 1996). In particular, DCs express high  
levels of mRNA for CCR1 (receptor for RANTES), CCR2 (receptor shared  
by MCP-1 and MCP-3), CCR3 (receptor for eotaxin) (Rubbert *et al.*,  
1998), CCR5 (receptor for MIP-1 $\alpha$ , MIP-1 $\beta$ , and RANTES) (Sozzani *et al.*,  
1995), and CCR6 (receptor for MIP-3 $\alpha$ ) (Greaves *et al.*, 1997; Power  
*et al.*, 1997). CCR1, CCR5, and CCR6, which are expressed on DC<sub>imm</sub>,  
are down-regulated during maturation (Sozzani *et al.*, 1998; Dieu *et al.*,  
1998). Conversely, CCR7, a receptor for MIP-3 $\beta$ , is lacking on DC<sub>imm</sub>  
but is induced upon activation (Dieu *et al.*, 1998; Sozzani *et al.*, 1998).  
Importantly, MIP-3 $\alpha$  is preferentially produced at sites enriched with  
DC<sub>imm</sub> whereas MIP-3 $\beta$  is preferentially expressed within the paracortex  
of secondary lymphoid organs where DC<sub>mat</sub> migrate (Dieu *et al.*, 1998).  
Thus the coordinated expression of distinct chemokine receptors may play  
a critical role in the migration of DCs at various stages of maturation.

The migration of DCs induced by bacteria is likely due to the capacity  
of LPS to stimulate many cell types to secrete cytokines and chemokines  
that modulate DC movement and maturation. These include M-CSF (Heu-  
fler *et al.*, 1988; Witmer-Pack *et al.*, 1987), TNF- $\alpha$  (Sallusto *et al.*, 1995;  
Sallusto and Lanzavecchia, 1994), IL-1 (Koide *et al.*, 1987), MIP-1 $\alpha$ , -1 $\beta$ ,  
and -1 $\gamma$  (Mohamadzaheh *et al.*, 1996; Sozzani *et al.*, 1996).

Another heterogeneous multifunctional molecule involved in DC traf-  
ficking is CD44 (Weiss *et al.*, 1997). CD44 is a receptor for the extracellular  
matrix component hyaluronate, which is involved in lymphocyte homing  
and activation as well as spreading of tumor metastases. CD44 is encoded  
by a total of 20 exons, 7 of which form the invariant extracellular region  
of the so-called standard form (CD44s). By alternative splicing, up to  
10 variant exons (CD44v1-v10) can be inserted into the cell membrane  
(Herrlich *et al.*, 1993). The CD44 isoforms play an essential role in LC and  
DC functions, the CD44 isoforms being differentially modulated during the  
LC-dependent sensitization phase of contact hypersensitivity, LC activa-

tion, and migration from the skin, and DC adhesion to the paracortical T cell zones of peripheral lymph nodes. During their migration to peripheral lymph nodes, LCs and DCs up-regulate pan-CD44 epitopes and sequences encoded by CD44 variant exons CD44v4, v5, v6, and v7 (Weiss *et al.*, 1997).

#### 4. Costimulatory Molecules

The most reliable functional assessment of histocompatibility remains the mixed lymphocyte reaction (MLR), in which T cells proliferate in response to allogeneic antigen-presenting cells (APCs). DCs are at least 30- to 100-fold more efficient than other APC populations, including B cells and macrophages, in inducing the MLR (Steinman and Witmer, 1978; Van Voorhis *et al.*, 1983). Numerous cytokines, including IL-12, IL-4, and IFN- $\gamma$  are released when DCs stimulate T cells in the MLR. Although CD4<sup>+</sup> cells account for much of the T cell proliferation during the MLR, DCs can also stimulate CD8<sup>+</sup> T cells without CD4<sup>+</sup> help, although higher antigen-presenting cell doses are needed (Inaba *et al.*, 1987; Young and Steinman, 1990). This implies that either antigen-presenting cells are killed during the course of the response or that stimulation is simply less efficient in the absence of CD4 help. DCs are also 10- to 50-fold more potent than monocytes or B cells in inducing T cell responses to femtomolar concentrations of superantigens (Bhardwaj *et al.*, 1992, 1993). However, the unique and most critical function of DCs is their ability to prime naive T cells to proteins that require processing into peptides (Christinck *et al.*, 1991; Croft *et al.*, 1992).

Antigen-loaded DCs and antigen-specific T cells form aggregates that constitute a microenvironment optimal for the development of an immune response (Flechner *et al.*, 1988; Inaba and Steinman, 1984). The interaction between DCs and T cells is coordinated by several molecules. "Signal one" is represented by MHC-peptide complexes and is recognized by antigen-specific TCRs. The availability of TCR transgenic mice has allowed investigators to prove that the capacity of DCs to induce a primary antigen-specific T cell response to soluble antigens *in vitro* is 100- to 300-fold more efficient than that of any other APC (Croft *et al.*, 1992; Macatonia *et al.*, 1995).

High levels of adhesins ICAM-1 (CD54), ICAM-3 (CD50), LFA-3 (CD58), and  $\beta_1$  integrin (CD29), and cell binding and homing molecules LFA-1 (CD11a), LFA-2 (CD2), and LFA-3, enhance adhesion and signaling (Caux *et al.*, 1994c; Freudenthal and Steinman, 1990; Larsen *et al.*, 1992; Lenz *et al.*, 1993; Young *et al.*, 1992). A variety of accessory molecules, coexpressed on DCs (B7.1/CD80, B7.2/CD86, CD40) and interacting with ligands and counterreceptors on T cells, together constitute "signal two," which is required to initiate T lymphocyte activation. Studies with antibod-

DC adhesion to the paracortical T during their migration to peripheral pan-CD44 epitopes and sequences 4, v5, v6, and v7 (Weiss *et al.*, 1997).

### Key Molecules

ment of histocompatibility remains 1), in which T cells proliferate in ting cells (APCs). DCs are at least her APC populations, including B MLR (Steinman and Witmer, 1978; tokines, including IL-12, IL-4, and ate T cells in the MLR. Although cell proliferation during the MLR, outh CD4<sup>+</sup> help, although higher led (Inaba *et al.*, 1987; Young and r antigen-presenting cells are killed at stimulation is simply less efficient e also 10- to 50-fold more potent ig T cell responses to femtomolar dwaj *et al.*, 1992, 1993). However, f DCs is their ability to prime naive ing into peptides (Christinck *et al.*,

pecific T cells form aggregates that for the development of an immune d Steinman, 1984). The interaction l by several molecules. "Signal one" lexes and is recognized by antigen-transgenic mice has allowed investi- DCs to induce a primary antigen- tigen *in vitro* is 100- to 300-fold APC (Croft *et al.*, 1992; Macatonia

CD54), ICAM-3 (CD50), LFA-3 cell binding and homing molecules 7A-3, enhance adhesion and signal- and Steinman, 1990; Larsen *et al.*, 2). A variety of accessory molecules, (CD86, CD40) and interacting with s, together constitute "signal two," te activation. Studies with antibod-

ies using human and mouse DCs have shown that CD86 on DCs is so far the most critical molecule for amplification of T cell responses (Caux *et al.*, 1994c; Inaba *et al.*, 1995). The interaction between CTLA-4/CD28 on T cells, and CD80/CD86 on DCs, also appears to play a role in the regulation of type 1 versus type 2 T cell development. In particular, B7.1/CD80 rather orients toward type 1 responses, whereas B7.2/CD86 ligation rather skews toward type 2 responses (Freeman *et al.*, 1995; Kuchroo *et al.*, 1995).

### 5. Signaling of DCs

#### Members of the TNF/TNF Receptor Families.

1. *TNF and TNF-R.* The effects of TNF on DC progenitors were identified in the early 1990s (Caux *et al.*, 1992a; Reid *et al.*, 1992; Santiago-Schwarz *et al.*, 1992). TNF enhances DC development through several mechanisms. In particular, TNF allows primitive hematopoietic progenitor cells to respond to IL-3 and GM-CSF following up-regulation of the  $\beta$  chain common to the IL-3/IL-5/GM-CSF receptor (Caux *et al.*, 1992b, 1993; Sato *et al.*, 1993). Furthermore, TNF inhibits granulopoiesis (Caux *et al.*, 1993), possibly by decreasing G-CSF-R expression. TNF- $\alpha$  is particularly important in the final maturation of these cells and the effects appear to be mostly mediated through TNF-R1/p55/CD120a (Lardon *et al.*, 1997), although TNF-R2/p75/CD120b has been identified on DCs (McKenzie *et al.*, 1995).

2. *CD40/CD40L.* The CD40 molecule, a member of the TNF-R family, is found on the surface of B lymphocytes, dendritic cells, hematopoietic progenitor cells, epithelial cells, and carcinomas (reviewed in Banchereau *et al.*, 1994; Grewal *et al.*, 1997; Van Kooten and Banchereau, 1996). The natural ligand for CD40 (CD40L/CD154) is expressed on the surface of activated CD4<sup>+</sup> and CD8<sup>+</sup> T cells, basophils, and B cells, as well as on DCs. Although the DC-T interaction has been traditionally viewed as a one-way interaction whereby DCs activate T cells, there is now evidence that T cells may play an important role in activating DCs via CD40L-CD40 interactions (Fig. 6). This further enhances the T cell stimulatory capacity of DCs. Ligation of CD40 also increases DC viability (Caux *et al.*, 1994b; Ludwig *et al.*, 1995) and induces DC maturation manifested by increased expression of CD80, CD83, and CD86 (Caux *et al.*, 1994b; Sallusto and Lanzavecchia, 1994). Following CD40 ligation DCs produce numerous cytokines, including IL-1, TNF, chemokines, and, importantly, IL-12, a key cytokine for the generation of Th1 responses (Cella *et al.*, 1996; Macatonia *et al.*, 1995). It is commonly accepted that macrophages represent the main source of IL-12 during immune responses to pathogens (Caux *et al.*, 1993; Skeen *et al.*, 1996; Takahashi *et al.*, 1993; Trinchieri, 1995),

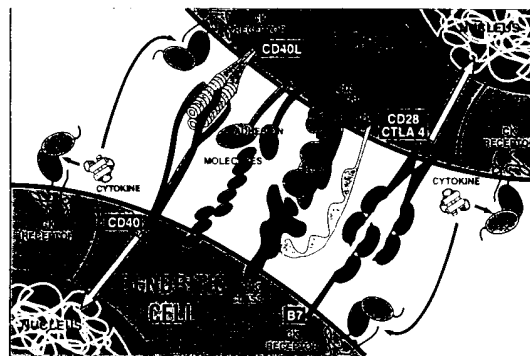
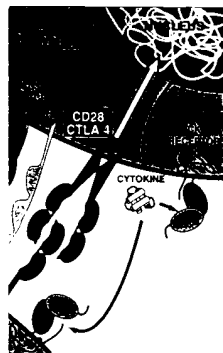


FIG. 6. *The dialogue between dendritic cells and T cells.* CD4<sup>+</sup> T cells recognize peptide presented by class II MHC on dendritic cells. Adhesion molecules strengthen the interaction. This results in up-regulation of CD40 ligand on T cells. Triggering of CD40 on DCs permits cytokine production and up-regulation of CD80/CD86 (B7). The secreted cytokines further activate T cells and support their proliferation. The increased CD80/CD86 expression on DCs triggers CD28 and/or CTLA-4 on T cells. The T cells then secrete cytokines in turn, which will either further activate the DCs or act as autocrine T cell growth factors.

but studies with *Toxoplasma* and *Leishmania* suggest that DCs may indeed be the first cells to make IL-12 during an immune response (Gorak *et al.*, 1998; Sousa *et al.*, 1997). The production of chemokines may be important to recruit other antigen-specific cells (e.g., CD8<sup>+</sup> T cells or B cells), whereas TNF may induce the apoptosis of nonspecific bystander T and B cells or act as an autocrine agent to keep the DCs in an activated state.

Recently, CD40L-activated DCs were found to express decysin, a novel member of the disintegrin metalloproteinases, which include the enzymes that cleave the transmembrane TNF precursor into soluble TNF (Black *et al.*, 1997; Moss *et al.*, 1997). Interestingly, decysin appears to be expressed by the mature myeloid/nonlymphoid DCs in germinal centers (Grouard *et al.*, 1996) but not by those in the T cell areas. A few molecules are down-regulated in response to CD40 activation. Among these are CD1a and the recently isolated DORA, a member of the CD8 family of receptors whose function on DC remains to be determined (Bates, 1998).

The importance of CD40-dependent activation of DCs is illustrated in the hyper-IgM syndrome of humans and mice with congenital and experimental alterations of CD40L, respectively. These individuals display a syndrome more suggestive of a primary T cell deficit than a primary B cell deficit. In particular, they show considerably altered T cell priming that results in increased susceptibility to numerous pathogens (e.g., *Leishmania*, *Pneumocystis*) (Grewal *et al.*, 1997).



*l* T cells. CD4<sup>+</sup> T cells recognize peptide ion molecules strengthen the interaction. cells. Triggering of CD40 on DCs permits D86 (B7). The secreted cytokines further ie increased CD80/CD86 expression on e T cells then secrete cytokines in turn, is autocrine T cell growth factors.

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The CD40-activated DCs can trigger T killer responses *in vitro* and *in vivo* in the absence of helper T cells (Bennett *et al.*, 1998; Ridge *et al.*, 1998; Schoenberger *et al.*, 1998). As stated by Ridge *et al.* (1998), a conditioned DC can be a temporal bridge between a CD4<sup>+</sup> T helper and a T killer cell. However, CD40 activation of DCs can be bypassed by inflammatory agents, as provided by an adjuvant (Bennett *et al.*, 1998) or by viral infection (Ridge *et al.*, 1998). Interestingly, LCs from CD40L<sup>-/-</sup> mice fail to emigrate to the draining lymph node on antigen sensitization, resulting in decreased contact hypersensitivity (Flores-Romo, personal communication). Of note, CD40L has also been identified on activated DCs (Pinchuk *et al.*, 1996), though its role remains to be determined.

3. *OX40/OX40L*. Mature DCs constitutively express OX40L, the ligand for OX40, which is another member of TNF-R family present on activated peripheral CD4<sup>+</sup> T cells and a subset of CD8<sup>+</sup> T cells. Ligation of OX40L on monocyte-derived DCs, which are at an intermediate and reversible stage of maturation, markedly enhances their development into typical mature DCs (Ohshima *et al.*, 1997). Engagement of T cell CD40 promotes the development of anti-CD3-stimulated naive T cells into Th2 effectors producing high levels of IL-4, IL-5, and IL-13, but little IFN- $\gamma$ . Conversely, blocking OX40/OX40L interaction in primary cultures containing naive T cells and allogeneic DCs, using anti-OX40L monoclonal antibodies, inhibits the development of IL-4/IL-5-secreting T cells (Oshima and Delespesse, Santa Fe, 1998).

4. *RANK-L/TRANCE/ODF and RANK/TRANCE-R/osteoprotegerin*. A new member of TNF-R family derived from dendritic cells, RANK (receptor activator of NF- $\kappa$ B)/TRANCE-R (TNF-related activation-induced cytokine), and its ligand RANK-L/TRANCE, have been isolated and characterized (Anderson *et al.*, 1997; Wong *et al.*, 1997). RANK-L/TRANCE expression is restricted to lymphoid organs and T cells (Wong *et al.*, 1997). High levels of RANK/TRANCE-R are detected on mature DCs but not on freshly isolated B cells, T cells, or macrophages. RANK/TRANCE-R signals via TNF receptor-associated factor 2 (TRAF2) and increases DC survival by up-regulating bcl-x<sub>L</sub> expression, thereby providing another tool to enhance DC activity by prolonging viability (Wong *et al.*, 1997). RANK/TRANCE-R augments the ability of DCs to stimulate naive T cell proliferation in the MLR and increases the survival of RANK-L/TRANCE-positive T cells generated with IL-4 and TGF- $\beta$  (Anderson *et al.*, 1997; Wong *et al.*, 1997).

More recently, the osteoclast differentiation factor (ODF) was found to be identical to TRANCE/RANK-L (Yasuda *et al.*, 1998). This cytokine is present on the surface of stromal cells and is responsible for osteoclast differentiation. Osteoprotegerin (OPG), a molecule of the TNF-R family

that suppresses bone resorption (Simonet *et al.*, 1997), binds to TRANCE/RANK-L/ODF, thereby inhibiting osteoclast differentiation (Suda *et al.*, 1995). Thus TRANCE/RANK-L/ODF appears to bind to two distinct molecules of the same family: RANK and OPG. However, distinct from the other agonist receptor-ligand pairings in this family (TNF, LT $\alpha$ , LT $\beta$  and TNF-R1, TNF-R2), OPG acts as a soluble competitive inhibitor of the transmembrane receptor RANK.

5. *Fas/FasL*. Fas/Apo 1 (CD95) is expressed on human DCs generated *in vitro* by culturing CD34<sup>+</sup> HPCs with GM-CSF and TNF- $\alpha$ , and on Fas ligation DCs undergo apoptosis (Bjorck *et al.*, 1997a). Surprisingly and in contrast to B cells (Garrone *et al.*, 1995), fully mature DCs obtained after CD40 ligation are fully resistant to Fas ligation, possibly as a consequence of up-regulated bcl-2 expression. Parallel experiments with mature, interdigitating DCs isolated from tonsils have revealed that IDCs express Fas but do not enter apoptosis after Fas ligation, a finding correlating with their high level of bcl-2 (Bjorck *et al.*, 1997a). Other mechanisms should therefore be pursued to explain the *in vivo* disappearance of antigen-loaded, mature DCs during an immune response (Ingulli *et al.*, 1997). Studies in mice have also shown that DCs of the lymphoid lineage express a FasL (Lu *et al.*, 1997; Suss and Shortman, 1996), which may be distinct from the classical one (K. Shortman, personal communication).

#### 6. Enzymes

Because of their potent antigen-presenting capacity, DCs are expected to express an enzymatic armamentarium tailored to the degradation of virtually any antigen into peptides. These antigens include not only proteins but also large particles such as viruses, bacteria, mycobacteria, parasites, and apoptotic bodies. Therefore, their processing undoubtedly requires a very diverse set of enzymes, but little has been published to date on this topic. Cathepsin D, an asparagyl protease, has been identified within human and murine DCs (Lutz *et al.*, 1997; Sallusto *et al.*, 1995). Furthermore, a novel member of the disintegrin metalloproteinases, decysin, has been identified using cDNA subtraction libraries (Mueller *et al.*, 1997b). Although absent from DC<sub>pre</sub> and DC<sub>imm</sub>, decysin is induced to high levels following spontaneous and CD40-induced maturation. *In vivo*, decysin appears restricted to germinal center dendritic cells (Grouard *et al.*, 1996), but its functions remain unknown. As discussed earlier, type IV collagenase, identified in Langerhans cells, facilitates the migration of these cells across basement membranes. Genomic analysis of DC libraries has also permitted the identification of numerous protease inhibitors. In particular, several cystatins, which are inhibitors of cysteine proteases, have been identified (S. Lebecque, C. Caux, and G. Zurawski, personal communication). A

*t al.*, 1997), binds to TRANCE/ast differentiation (Suda *et al.*, appears to bind to two distinct OPG. However, distinct from in this family (TNF, LT $\alpha$ , LT $\beta$  soluble competitive inhibitor of

essed on human DCs generated M-CSF and TNF- $\alpha$ , and on Fas *al.*, 1997a). Surprisingly and in ally mature DCs obtained after tion, possibly as a consequence xperiments with mature, inter-evealed that IDCs express Fas ion, a finding correlating with 7a). Other mechanisms should *ivo* disappearance of antigen-response (Ingulli *et al.*, 1997). of the lymphoid lineage express i, 1996), which may be distinct nal communication).

ng capacity, DCs are expected tailored to the degradation of tigen include not only proteins eteria, mycobacteria, parasites, essing undoubtedly requires a been published to date on this is been identified within human o *et al.*, 1995). Furthermore, a proteinases, decysin, has been es (Mueller *et al.*, 1997b). Al-ysin is induced to high levels l maturation. *In vivo*, decysin itic cells (Grouard *et al.*, 1996), sed earlier, type IV collagenase, migration of these cells across DC libraries has also permitted ibitors. In particular, several roteases, have been identified , personal communication). A

serine protease inhibitor (serpin) has also been identified from subtractive cDNA libraries (Mueller *et al.*, 1997a). This serpin is absent from mono-cytes, B cells, and T cells, but is expressed in CD40-activated DCs.

Additional studies demonstrate that proteases and protease inhibitors are also important in the presentation of antigens, most particularly in the routing of the class II MHC antigens within DCs. DCs express cathepsin S, an enzyme that has been shown to play a role in the processing of the invariant chain in B cell lines. Indeed, blocking cathepsin S with the specific and irreversible inhibitor LHVS (Riese *et al.*, 1996; Villadangos *et al.*, 1997) results in a significantly decreased export of class II MHC to the DC surface, while the total content remains unchanged. In DC<sub>imm</sub>, inefficient Ii chain cleavage due to low cathepsin S activity leads to the transport of class II MHC-Ii chain complexes to lysosomes. In contrast, elevated Cathepsin S activity in DC<sub>mat</sub> results in efficient transport of class II MHC molecules to the cell surface. The increased cathepsin S activity observed following DC maturation is not due to its increased transcription but to the decreased presence of its specific inhibitor, cystatin C, a cystein protease inhibitor (Henskens *et al.*, 1996; Pierre and Mellman, 1998); Maurer and Sting, personal communication).

#### 7. Natural Killer Phenotype of Dendritic Cells

Rat spleen and thymus dendritic cells express low levels of the natural killer cell receptor protein 1 (NKR-P1) (Josien *et al.*, 1997). NKR-P1, a disulfide-linked homodimer expressed by all NK cells and a small subset of T cells, belongs to group V of the C-type lectin superfamily. This superfamily also includes the CD69, Ly-49, and CD54 molecules (Lanier, 1997; Moretta and Moretta, 1997). The rat NKR-P1 molecule is an activation receptor that leads to stimulation of granule exocytosis. The expression of NKR-P1 on DCs is strongly up-regulated after overnight culture. In addition to expressing this typical NK cell marker, rat spleen DCs, but not thymus DCs, are able to kill the NK cell-sensitive target YAC-1. Human dendritic cells generated *in vitro* by culturing monocytes or CD34<sup>+</sup> HPCs also express NKR-P1, ligation of which results in Ca<sup>2+</sup> fluxes and IL-12 secretion (Poggi *et al.*, 1997). It is not presently known whether human DCs express any functional NK activity.

#### 8. Calcium Channels of Dendritic Cells

Monocyte-derived DCs display L-type calcium channels that mediate the influx of extracellular Ca<sup>2+</sup> (Poggi *et al.*, 1998a). These Ca<sup>2+</sup> channels are composed of three transmembrane subunits ( $\alpha$ 1C,  $\gamma$ , and  $\alpha$ 2 $\delta$  complex) and one cytoplasmic chain (the  $\beta$ 1 chain) (Catterall and Striessnig, 1992), comparable to those of skeletal and cardiac muscle. The dihydropyridine

derivative nifedipine, which specifically binds to the  $\alpha 1C$  chain, prevents apoptotic body engulfment and IL-12 secretion by DCs (Poggi *et al.*, 1998b). Importantly, HIV-1 Tat also blocks these two DC functions (Zocchi *et al.*, 1997) by acting on these  $Ca^{2+}$  channels (Poggi *et al.*, 1998a). This may explain the altered function of circulating DCs in AIDS patients (Macatonia *et al.*, 1989).

### III. Ontogeny of Dendritic Cells

Studies of DCs have been greatly hampered in the past by difficulties in isolating these cells from tissues or blood in substantial numbers and with purity. Great progress has been achieved since the establishment of several procedures for the *in vitro* generation of murine and human DCs from progenitors in bone marrow, placental and umbilical cord blood, and cytokine-mobilized peripheral blood (Caux *et al.*, 1992a, 1996a; Flores-Romo *et al.*, 1997; Inaba *et al.*, 1992a,b; Santiago-Schwarz *et al.*, 1992; Strunk *et al.*, 1996; Szabolet *et al.*, 1995, 1996; Young *et al.*, 1995). Methods have also been developed to generate DCs from blood monocytes (Bender *et al.*, 1996; Reddy *et al.*, 1997; Romani *et al.*, 1994, 1996; Sallusto and Lanzavecchia, 1994). The current understanding of DC ontogeny is summarized in Fig. 7.

#### A. GENERATION OF MOUSE DENDRITIC CELL LINES

Addition of GM-CSF to mouse blood or bone marrow results in the formation of DC aggregates that originate from Ia-negative nonadherent cells (Inaba *et al.*, 1992b, 1993). Long-term dendritic cell lines have also been generated from fetal tissues using either GM-CSF (Winzler *et al.*, 1997) or stromal cell culture supernatants (Takashima *et al.*, 1995).

#### B. GENERATION OF DENDRITIC CELLS FROM CD34<sup>+</sup> HEMATOPOIETIC PROGENITOR CELLS

##### 1. *TNF, in Association with GM-CSF or IL-3, Induces Development of DCs from CD34<sup>+</sup> HPCs*

CD34<sup>+</sup> HPCs, isolated from cord blood or bone marrow mononuclear cells, can be induced to proliferate *in vitro* in response to several cytokines in combinations. TNF strongly potentiates the proliferation of CD34<sup>+</sup> HPCs induced by either IL-3 or GM-CSF (Caux *et al.*, 1990, 1992a; reviewed in Caux and Banchereau, 1996). After 12 days, a majority of cells express CD1a and acquire typical DC features according to morphology, phenotype (CD40, CD4, CD54, CD80, CD86; high levels of class II MHC, lack of CD64 and CD35), presence of Birbeck granules (specific for LCs) in 20% of cells, and potent capacity to induce proliferation of naive T cells

inds to the  $\alpha 1C$  chain, prevents secretion by DCs (Poggi *et al.*, 1998a). These two DC functions (Zocchi *et al.*, 1998a). This regulating DCs in AIDS patients

### Dendritic Cells

pered in the past by difficulties in obtaining substantial numbers and have been established since the establishment of murine and human DCs from fetal liver and umbilical cord blood, and from human peripheral blood monocytes (Bender *et al.*, 1994, 1996; Sallusto and Lanzetta, 1996). Methods for generating DCs from blood monocytes (Bender *et al.*, 1994, 1996; Sallusto and Lanzetta, 1996) and of DC ontogeny is sum-

### CELL LINES

or bone marrow results in the generation of Ia-negative nonadherent dendritic cell lines have also been established with GM-CSF (Winzler *et al.*, 1995; Takashima *et al.*, 1995).

### FROM CD34<sup>+</sup> HEMATOPOIETIC

#### IL-3, Induces Development of HPCs

For bone marrow mononuclear cells, in response to several cytokines, including IL-3, the proliferation of CD34<sup>+</sup> HPCs (Caux *et al.*, 1990, 1992a; Young *et al.*, 1995). After 12 days, a majority of cells are CD34<sup>+</sup> and CD11c<sup>+</sup> according to morphology, size, and high levels of class II MHC, Birbeck granules (specific for LCs) and induce proliferation of naive T cells

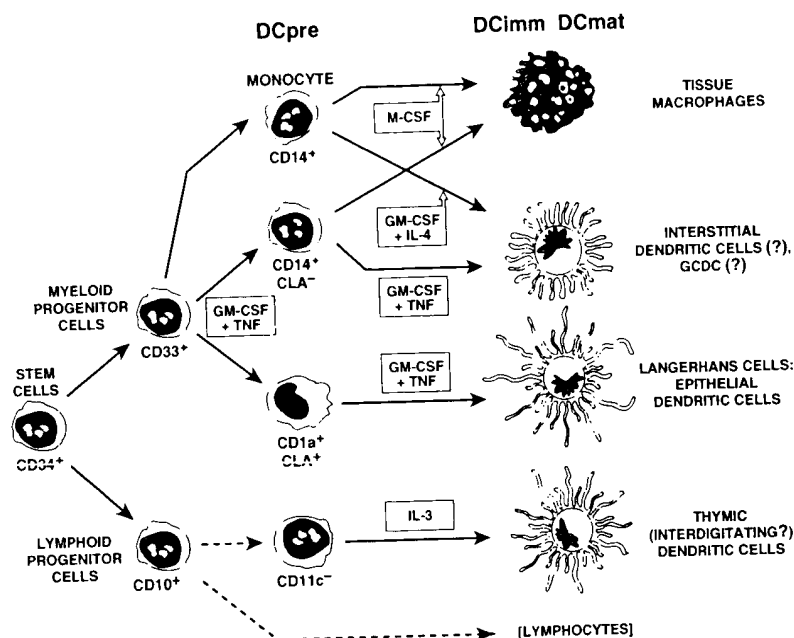


FIG. 7. *Dendritic cell ontogeny.* The pluripotent stem cell gives rise to myeloid and lymphoid progenitor cells. The lymphoid progenitor may give rise to the CD11c<sup>+</sup> DC precursor that may differentiate into thymic dendritic cells, as well as a subpopulation of parafollicular, interdigitating dendritic cells in the T cell-rich areas of secondary lymphoid tissues. The myeloid DC progenitor differentiates *in vitro* into a CD1a<sup>+</sup> precursor that yields Langerhans cells and a CD14<sup>+</sup> precursor that may yield germinal center DCs. The *in vitro*-generated CD14<sup>+</sup> precursor may be the equivalent of circulating monocyte precursors of DCs, although IL-4 has not proved to be as critical in the DC differentiation of the former.

to alloantigen and of CD4<sup>+</sup> T cells to soluble antigen (Caux *et al.*, 1992a,b, 1995, 1996b). TNF is also required for the clonogenic growth of pure human DC colonies in the additional presence of GM-CSF (Young *et al.*, 1995). Addition of stem cell factor (SCF, *c-kit* ligand) and/or Flt-3L increases the yield of DCs but does not directly affect DC differentiation *in vitro* (Siena *et al.*, 1995; Strobl *et al.*, 1997; Young *et al.*, 1995). For cultures performed under serum-free conditions, TGF- $\beta$  may be required for generation of DCs with characteristics of LCs, e.g., Birbeck granules and Lag antigen (Riedl *et al.*, 1997; Strobl *et al.*, 1997). The maturation of CD34<sup>+</sup> HPCs into DCs also involves protein kinase C-mediated signaling and can be partly induced by phorbol esters alone (Davis *et al.*, 1998).

## 2. CD34<sup>+</sup> HPCs, Cultured in the Presence of GM-CSF + TNF, Differentiate along Two Independent DC Pathways

Many candidate DCs are CD1a<sup>+</sup> CD14<sup>+</sup> during the later stages of culture (approximately days 12–14) from CD34<sup>+</sup> HPCs in FCS-containing medium; CD1a expression is lost with final maturation, however, just as CD83 expression increases. When examined earlier, however, two DC subsets emerge independently by days 5–7, as defined by the exclusive expression of CD1a and CD14. Both precursor populations eventually mature into DCs in response to GM-CSF + TNF: CD1a<sup>+</sup> CD14<sup>+</sup> cells give rise to CD1a<sup>+</sup> CD14<sup>+</sup> LCs (Birbeck granules, Lag<sup>+</sup>, E-cadherin<sup>+</sup>), while CD1a<sup>+</sup> CD14<sup>+</sup> intermediates develop into interstitial (dermal) DCs that are also CD1a<sup>+</sup> CD14<sup>+</sup> (lack of Birbeck granules, Lag<sup>+</sup>, E-cadherin<sup>+</sup>, CD2<sup>+</sup>, CD9<sup>+</sup>, CD68<sup>+</sup>, Factor XIIIa<sup>+</sup>). The CD1a<sup>+</sup> CD14<sup>+</sup> intermediate is bipotential, however, in that it can alternatively differentiate into CD1a<sup>+</sup> CD14<sup>+</sup> macrophages on reculture without exogenous cytokines; M-CSF can enhance viability and support possibly one additional round of cell division (Caux *et al.*, 1996a; Szabo *et al.*, 1996). Primitive CD34<sup>+</sup> CD38<sup>+</sup> hematopoietic progenitors can also develop into interstitial DCs when cultured over thymic stromal monolayers in the absence of exogenous cytokines (Miralles *et al.*, 1998).

The commitment to either pathway may have already occurred at the level of the CD34<sup>+</sup> HPCs. For example, a minor population of CD34<sup>+</sup> HPCs, which can be increased by exposure to TNF, coexpresses CD86, and this CD34<sup>+</sup> HPC subset exhibits bipotential differentiation capacity into macrophages or dendritic cells (Ryncarz and Anasetti, 1998). CD34<sup>+</sup> CLA<sup>+</sup> cells also reportedly give rise *in vitro* to Langerhans cells, whereas CLA<sup>+</sup> precursors yield interstitial DCs (Strunk *et al.*, 1997). Although the two populations are equally potent in stimulating naive CD45RA<sup>+</sup> cord blood T cells, each also displays specific activities (Caux *et al.*, 1997). In particular, interstitial DCs demonstrate a potent and long-lasting antigen uptake activity (FITC-dextran or peroxidase) that is about 10-fold higher than that of Langerhans cells and is mediated by mannose receptors. The high efficiency of antigen capture by interstitial DCs correlates with the expression of nonspecific esterase activity, a tracer of the lysosomal compartment that is not observed in Langerhans cells. A striking difference between the two populations is also the unique capacity of interstitial DCs to induce naive B cells to differentiate into IgM-secreting cells in response to CD40 ligation and IL-2. Thus, although T cell priming is accomplished by both DC populations, one can envision that the two different pathways of DC development are preferentially specialized: (1) the Langerhans cell type, which would be mainly involved in cellular immune responses, and

### Effect of GM-CSF + TNF on the Differentiation of DC Pathways

During the later stages of culture, DCs in FCS-containing medium, however, just as CD83<sup>+</sup> DCs, however, two DC subsets are defined by the exclusive expression of markers that eventually mature into CD1a<sup>+</sup> CD14<sup>-</sup> cells (give rise to interstitial (dermal) DCs that are CD1a<sup>+</sup> Lag<sup>-</sup>, E-cadherin<sup>-</sup>, CD2<sup>+</sup>, CD14<sup>+</sup> intermediate is bipotential and differentiate into CD1a<sup>-</sup> CD14<sup>+</sup> cells in the presence of cytokines; M-CSF can initiate a second round of cell division to generate CD34<sup>+</sup> CD38<sup>-</sup> hematopoietic DCs when cultured in the presence of exogenous cytokines

have already occurred at the time of the minor population of CD34<sup>+</sup> cells to TNF, coexpresses CD86, and has a high differentiation capacity (Caux and Anasetti, 1998). CD34<sup>+</sup> cells are Langerhans cells, whereas CD34<sup>+</sup> cells are not (Jonuleit *et al.*, 1997). Although the expression of the late antigen CD45RA<sup>+</sup> is low in these cells (Caux *et al.*, 1997). In contrast to the long-lasting antigen CD45RO<sup>+</sup> that is about 10-fold higher in Langerhans cells than in interstitial DCs. The expression of CD45RO<sup>+</sup> correlates with the expression of the lysosomal marker CD63. A striking difference is the antigen-presenting capacity of interstitial DCs. M-CSF-secreting cells in response to antigen cell priming is accomplished at the two different pathways described: (1) the Langerhans cell pathway, which leads to cellular immune responses, and

(2) the interstitial DC, which would be dedicated to the initiation of cognate T cell help and humoral immune responses by B cells.

### C. GENERATION OF DENDRITIC CELLS FROM BLOOD MONONUCLEAR CELLS

Using GM-CSF and IL-4 (Cella *et al.*, 1997a; Chapuis *et al.*, 1997; Picot *et al.*, 1996; Porcelli *et al.*, 1992; Romani *et al.*, 1994, 1996; Sallusto and Lanzavecchia, 1994; Zhou and Tedder, 1996) or GM-CSF and IL-13 (Piemonti *et al.*, 1995), lymphocyte-depleted, adherent blood mononuclear cells as well as purified CD14<sup>+</sup> monocytes yield DCs that can be maintained in culture for weeks in the presence of cytokines and FCS-containing medium. The resulting DCs display features of DC<sub>imm</sub> (low levels of CD80, CD86, intracytoplasmic expression of MHC class II, an efficient antigen-capturing and -processing capacity, and a weak capacity to prime naive T cells) (reviewed in Caux and Banchereau, 1996). When stimulated by T cell signals such as CD40L (Sallusto *et al.*, 1995; Sallusto and Lanzavecchia, 1994) or signals activating ceramide mediators, such as LPS, TNF, and IL-1 (Sallusto *et al.*, 1996), these DCs undergo the phenotypic and functional changes of a maturation process. The irreversible maturation of these cells after culture in media with human serum or plasma necessitates addition of macrophage-conditioned medium that contains at least IL-1, IL-6, TNF, and IFN- $\alpha$  (Bender *et al.*, 1996; Reddy *et al.*, 1997; Romani *et al.*, 1996), and undoubtedly other as yet undefined inflammatory mediators such as prostaglandin (Jonuleit *et al.*, 1997a). Addition of TGF- $\beta$  to cultures of monocytes with GM-CSF + IL-4 results in the generation of DCs with properties of Langerhans cells (Geissmann *et al.*, 1998). Thus lymphocyte-depleted, peripheral blood mononuclear cells, i.e., monocytes, represent a considerable pool of circulating precursors of DCs and macrophages.

Surprisingly, highly purified neutrophil granulocyte-committed precursors can also be driven to acquire DC characteristics when cultured in the presence of GM-CSF + TNF- $\alpha$  + IL-4 (Oehler *et al.*, 1998). This indicates that cells from the innate immune system can be reprogrammed to become inducers of the adaptive immune system even at a penultimate stage of terminal differentiation.

### D. A LYMPHOID PATHWAY OF DENDRITIC CELL DEVELOPMENT

The myeloid or nonlymphoid model of DC development does not apply to the thymus, where DCs are indeed present to induce death of self-reactive thymocytes (Ardavin, 1997; Brouck *et al.*, 1997). Murine thymic DCs express a peculiar phenotype, with lymphoid cell markers such as the CD8  $\alpha\alpha$  homodimer, CD2, and BP1 (Vremec *et al.*, 1992; Wu *et al.*, 1995). A subgroup of these DCs is found in spleen (50% of DCs) as well

as lymph node (Inaba *et al.*, 1997). These cells originate from a progenitor cell that can also give rise to T cells and B cells (Ardavin *et al.*, 1993; Wu *et al.*, 1996). The differentiation of this precursor *in vitro* is independent of GM-CSF and can be achieved by combining TNF, IL-1, IL-3, IL-7, SCF, Flt-3L, and CD40L (Saunders *et al.*, 1996). Human CD34<sup>+</sup> HPCs have also been identified that can give rise to T cells, B cells, and DCs *in vitro* (Galy *et al.*, 1995; Res *et al.*, 1996). A mature human lymphoid DC, however, has not been identified to date. The function of these lymphoid DCs is discussed with regard to immune tolerance in Sections V.D and V.E.

#### E. FLT-3 LIGAND AND DENDRITIC CELLS

The search for new receptor tyrosine kinases led to the discovery of a murine gene termed fetal liver kinase 2 (*FLK-2*) and of a human gene termed FMS-like tyrosine kinase 3 (*FLT-3*), which are homolog. *FLT-3* also has substantial homology to *c-kit*, *c-fms*, and PDG receptor genes, which play a central role in hematopoiesis. *FLT-3* is expressed on early, nonerythroid hematopoietic progenitor cells, as well as more mature hematopoietic cells (Lyman and Jacobsen, 1998). Both human and murine ligands for *FLT-3* were cloned and have been shown to encode a type I transmembrane protein and a soluble protein following alternative splicing rather than proteolysis (Lyman and Williams, 1995). Flt-3L is found on various stromal cells and in a variety of tissues. *In vitro*, Flt-3L acts in concert with other cytokines to induce proliferation of early progenitors (Jacobsen *et al.*, 1995; Muench *et al.*, 1995; Shah *et al.*, 1996), but it has never been shown to have a differentiating capacity alone *ex vivo*. In contrast, *in vivo* administration of Flt-3L results in the blood recirculation of CD34<sup>+</sup> HPCs, and a striking enlargement of spleen, lymph nodes, and liver. The organs show increased levels of B cells but the most notable feature is an accumulation of dendritic cells (Maraskovsky *et al.*, 1996; Pulendran *et al.*, 1997; Shurin *et al.*, 1997).

#### F. CURRENT VIEW OF THE PATHWAYS OF DENDRITIC CELL DEVELOPMENT

Although DCs derive from proliferating CD34<sup>+</sup> progenitor cells, three stages of DC differentiation are being distinguished, namely, patrolling DC<sub>pre</sub>, tissue-residing DC<sub>imm</sub>, and DC<sub>mat</sub> from secondary lymphoid organs. DCs are also composed of distinct subpopulations, in many cases related to distinct precursors (Fig. 7). These precursors include CD4<sup>+</sup> CD14<sup>+</sup> monocytes, and CD4<sup>+</sup> CD14<sup>+</sup> CD11c<sup>-</sup> as well as CD4<sup>+</sup> CD14<sup>-</sup> CD11c<sup>+</sup> cells. Monocytes are primarily identified in the blood, whereas CD11c<sup>-</sup> and CD11c<sup>+</sup> precursors can be identified in blood and secondary lymphoid organs. CD11c<sup>-</sup> cells remain localized within T cell-rich areas, but CD11c<sup>+</sup>

cells originate from a progenitor cells (Ardavin *et al.*, 1993; Wu precursor *in vitro* is independent binding TNF, IL-1, IL-3, IL-7, (1996). Human CD34<sup>+</sup> HPCs to T cells, B cells, and DCs in mature human lymphoid DC, the function of these lymphoid in Sections V,D and V,E.

as

genes led to the discovery of a *FLK-2*) and of a human gene 3), which are homolog. *FLT-3* genes, and PDG receptor genes, *FLT-3* is expressed on early, as well as more mature hematopoietic cells (1988). Both human and murine have been shown to encode a type I receptor with following alternative splicing forms (1995). *Flt-3L* is found on various tissues. *In vitro*, *Flt-3L* acts on proliferation of early progenitors (Shah *et al.*, 1996), but it has no effect alone *ex vivo*. In the blood recirculation of spleen, lymph nodes, and bone marrow, B cells but the most notable cells (Maraskovsky *et al.*, 1996;

CD34<sup>+</sup> progenitor cells, three distinguished, namely, patrolling in secondary lymphoid organs. In many cases related to these precursors include CD4<sup>+</sup> CD14<sup>+</sup> as well as CD4<sup>+</sup> CD14<sup>+</sup> CD11c<sup>+</sup> in the blood, whereas CD11c<sup>+</sup> in the blood and secondary lymphoid organs and T cell-rich areas, but CD11c<sup>+</sup>

cells migrate into B cell follicles as germinal center DCs (not to be confused with follicular DCs). We currently think that the recirculating DC<sub>pre</sub> eventually colonize tissues to become DC<sub>imm</sub>. Although DCs are found in very low numbers in virtually every tissue, there is emerging evidence that epithelial DCs (Langerhans cells) and interstitial DCs represent alternative pathways of differentiation (Caux *et al.*, 1996a). The relationship between the above three stages of DC differentiation and these two populations of immature cells remains unresolved. It is possible that CD11c<sup>+</sup> precursors eventually differentiate into DCs within thymus and secondary lymphoid organs, where they establish and maintain tolerance. Blood and lymph also contain a very minor population of maturing DCs on their way from tissues to secondary lymphoid organs (reviewed in Banchereau and Steinman, 1998).

#### IV. Maturation of Dendritic Cells

##### A. STIMULATORS OF MATURATION

During migration after antigen loading, DCs undergo changes in phenotype and function as part of their maturation. This represents a control point for the onset of immunity. As discussed earlier, maturation includes a coordinate series of changes, which include down-regulation of macrophage receptors and Fc receptors, transition of the class II MHC-rich intracellular compartments to cell surface MHC peptide complexes, and the up-regulation of accessory molecules (Cella *et al.*, 1997b; Heufler *et al.*, 1988; Pierre *et al.*, 1997; Sallusto *et al.*, 1995; Witmer-Pack *et al.*, 1987; Yamaguchi *et al.*, 1997). A variety of agents contribute to DC maturation. These include cytokines such as IL-1, GM-CSF, and TNF- $\alpha$ , released by a variety of cell types, e.g., keratinocytes, mast cells, macrophages, or T cells, as well as other T cell products such as IL-2, and bacterial products such as LPS. Some viruses, e.g., influenza virus, can also directly induce the maturation of DCs ((Ridge *et al.*, 1998); Lanzavecchia, personal communication). Phagocytosed bacteria also induce DC maturation with an increased synthesis of MHC class I and class II molecules. In particular, bacteria stabilize MHC class I complexes and allow efficient loading of MHC class I molecules (Rescigno *et al.*, 1998). Intramembrane diffusible mediators such as ceramides, involved in transducing signals that originate from a variety of cell surface receptors, down-modulate antigen capture and thus mimic one step of DC maturation (Sallusto *et al.*, 1996). In this context, the potent DC maturation ability of LPS may be related to its structural similarity to ceramides. The transcription factors Rel/NF- $\kappa$ B proteins (p50, p52, p65, c-Rel, Rel-B) play an important role in the biology of DCs, from their ontogeny to their maturation. Physiologically high levels

of p50, p52, and Rel-B are restricted to accessory cells of the immune system, which include DCs and macrophages in the T cell zones (Carrasco and Bravo, 1993; Feuillard *et al.*, 1996). Studies have localized Rel-B to interdigitating DCs in lymph nodes as well as scattered germinal center cells, but not to undifferentiated DCs in normal skin (Pettit *et al.*, 1997). Active nuclear Rel-B has been detected by supershift assay only in differentiated DCs derived from either blood precursors or monocytes, and in B cells, implying that Rel-B may specifically transactivate genes within the nucleus that are critical for APC function (Pettit *et al.*, 1997). Rel-B knockout mice have no DCs in their altered lymphoid organs, although Langerhans cells are present (Burkly *et al.*, 1995; Salomon *et al.*, 1994). It is not clear whether the lack of DCs within secondary lymphoid organs results from altered cell migration, cell survival, or cell maturation.

#### B. IL-10 AS AN INHIBITOR OF DENDRITIC CELL MATURATION

Early studies have shown that IL-10 inhibits the antigen-presenting capacity of monocytes/macrophages (de Waal Malefyt *et al.*, 1992; Moore *et al.*, 1993). Subsequently, IL-10 was shown also to inhibit the APC functions of *in vitro*-generated DCs (Caux *et al.*, 1994a; Steinbrink *et al.*, 1997; Thomssen *et al.*, 1995) as well as freshly isolated tonsillar DCs and epidermal LCs (Caux *et al.*, 1994a; Peguet-Navarro *et al.*, 1994), through mechanisms that have not been fully established. Some studies indicate an inhibition of CD80 and CD86 expression (Buelens *et al.*, 1995; Mitra *et al.*, 1995; Ozawa *et al.*, 1996; Steinbrink *et al.*, 1997). Others fail to identify any alteration of CD80/CD86 or class II MHC peptide expression (Morel *et al.*, 1997). The lack of consensus is likely to stem from major differences in experimental protocols and differential sensitivity of DCs to IL-10 with regard to stage of maturation. In this respect, IL-10 inhibits DC expression of CD83 and CD86, as well as secretion of IL-8 and TNF when DCs have been activated with LPS; IL-10 does not have the same effect when DCs are terminally matured by exposure to CD40L (Buelens *et al.*, 1997a). Furthermore, the assays used to measure DC function should be carefully assessed, because the alterations may be very subtle. For example, IL-10-treated DCs appear to induce the differentiation of naive T cells toward the Th2 pathway (Allavena *et al.*, 1998; De Smedt *et al.*, 1997; Liu *et al.*, 1997a).

One of the most critical points of action by IL-10 on DCs concerns their ontogeny: IL-10 has been shown to inhibit the IL-4 + GM-CSF-induced proliferation of monocytes into DCs (Buelens *et al.*, 1997b; More *et al.*, 1997), to the benefit of macrophages (Allavena *et al.*, 1998). In unpublished studies (F. Rousset, C. Caux, and J. Banchemau) we also found that IL-10 could prevent the GM-CSF + TNF-dependent generation of DCs

accessory cells of the immune system in the T cell zones (Carrasco). Studies have localized Rel-B to all as scattered germinal center normal skin (Pettit *et al.*, 1997). supershift assay only in different-cursors or monocytes, and in B  $\gamma$  transactivate genes within the Pettit *et al.*, 1997). Rel-B knock-phoid organs, although Langer-; Salomon *et al.*, 1994). It is not onary lymphoid organs results or cell maturation.

#### C CELL MATURATION

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IL-10 does not have the same y exposure to CD40L (Buelens to measure DC function should tions may be very subtle. For lence the differentiation of naive i *et al.*, 1998; De Smedt *et al.*,

by IL-10 on DCs concerns their : the IL-4 + GM-CSF-induced lens *et al.*, 1997b; More *et al.*, ana *et al.*, 1998). In unpublished herean) we also found that IL-dependent generation of DCs

from CD34<sup>+</sup> HPCs. IL-10 acts as an antagonist to TNF, facilitating the generation of granulocytes and inducing the apoptosis of emerging DCs. IL-10-induced apoptosis of DCs has indeed been described with freshly isolated LCs (Ludewig *et al.*, 1995). This IL-10 inhibition of DC generation has been further demonstrated *in vivo*, where IL-10-expressing tumor cells blocked GM-CSF-induced accumulation of DCs (Qin *et al.*, 1997).

### V. Interactions of Dendritic Cells with T Cells

#### A. ASSOCIATION OF DENDRITIC CELLS WITH T CELLS *IN VIVO*

Substantial numbers of DCs are found in the T cell areas of secondary lymphoid tissues, where they are termed interdigitating DCs (IDCs). These IDCs form a network through which T cells continually recirculate. These DCs, which express mature epitopes that include CD80, CD83, and CD86, are nevertheless heterogeneous, as best illustrated in studies of mouse spleen and lymph nodes. Here the T cell areas are enriched in CD8 $\alpha\alpha$ <sup>+</sup> lymphoid DCs, and the CD8<sup>-</sup> myeloid DCs are mostly localized within the marginal zone (Pulendran *et al.*, 1997).

New observations that DCs within the T cell areas also express high levels of self-antigens and functional Fas-ligand capable of inducing CD4 T cell death suggest the presence of at least two sets of DCs in the T cell areas: (1) a migratory myeloid pathway that brings antigens from the periphery and induces immunity and (2) a lymphoid pathway that presents self-antigens and maintains tolerance (Steinman *et al.*, 1997). The heterogeneity of the DC population in animals is also illustrated in TGF- $\beta$ <sup>-/-</sup> mice that lack Langerhans cells and a subpopulation of Ep-Cam<sup>+</sup> DCs within lymph nodes (Borkowski *et al.*, 1996). Similarly clear distinctions have not yet been identified in human tissues.

DCs in the periphery acquire antigens and migrate to the T cell areas to initiate immunity. Although many *in vitro* and *in vivo* experiments argue strongly for the critical role of DCs in initiating immune responses, formal *in vivo* evidence has only recently been discovered. For example, proliferating T cells have been identified in contact with the DCs of the T cell-rich areas of secondary lymphoid organs after injection of either allogeneic cells (Kudo *et al.*, 1997), superantigens (Luther *et al.*, 1997), or protein antigen (Ingulli *et al.*, 1997). This last study used adoptive transfer of fluorochrome-labeled, ovalbumin-loaded DCs and T cells expressing a receptor specific for an OVA peptide-MHC complex. This interaction results in the expansion of antigen-specific T cells that peaks at 96 hr, even though antigen-pulsed DCs disappear after 48 hr. The likely elimination of antigen-loaded DCs represents an efficient way to limit the development of T cell responses.

The survival of mature CD4 T cells is also dependent on the presence of MHC class II-positive DCs (Brocker, 1997). After grafting of MHC class II-positive embryonic thymic tissue depleted of bone marrow-derived cells, an accumulation of CD4 T cells in the blood and secondary lymphoid organs can be observed only in mice expressing class II MHC on DCs but not in mice completely deficient in class II MHC (Brocker, 1997).

#### B. DENDRITIC CELLS EXPRESS CYTOKINES THAT ATTRACT T CELLS

To attract and select antigen-specific T cells, DC<sub>mat</sub> secrete multiple chemokines, including RANTES, MIP-1 $\alpha/\beta/\gamma$ , and IL-8. Novel chemokines are presently being identified using DC cDNA libraries. In particular, human dendritic cells present in the germinal center and T cell areas of secondary lymphoid organs express high levels of DC-CK1, which, in contrast to RANTES, MIP-1 $\alpha$ , and IL-8, preferentially attracts naive CD45RA<sup>+</sup> T cells (Adema *et al.*, 1997). DC<sub>mat</sub> from the T cell-rich areas secrete MIP-3 $\beta$ , which attracts naive CD4<sup>+</sup> T cells and CD8 cells (Ngo *et al.*, 1998). Thymic dendritic cells also express TECK, a novel CC chemokine that may be involved in T cell development (Vicari *et al.*, 1997). Finally, DCs also secrete IL-15, which is able to chemoattract T lymphocytes (Jonuleit *et al.*, 1997b).

#### C. DENDRITIC CELLS CAN DIRECTLY PRIME CD8<sup>+</sup> T CELLS

DCs can stimulate an MLR from highly purified CD8<sup>+</sup> T cells, though higher numbers of APCs are needed when compared to the response of CD4<sup>+</sup> T cells (Inaba *et al.*, 1997; Young and Steinman, 1990). Because allospecific CTLs are generated rapidly during these cultures, the need for higher APC numbers may indicate that the APCs are killed during the course of the response. Indeed there is now ample evidence that DCs represent excellent CTL targets. Alternatively and as discussed earlier, this lower efficiency may be due to a suboptimal maturation of the DCs because of the lack of helper T cell activation, mostly dependent on CD40 ligation (Bennett *et al.*, 1998; Ridge *et al.*, 1998; Schoenberger *et al.*, 1998). The availability of DCs devoid of class II MHC has facilitated confirmation of this unique functional property of DCs to prime CD8<sup>+</sup> cells independently of CD4<sup>+</sup> help. CD8<sup>+</sup> T cells specific for alloantigens, as well as for tumor and viral antigens, can be generated by DCs. In particular, class II MHC<sup>+</sup> class II MHC<sup>-</sup> epidermal Langerhans cell lines derived from fetal skin can activate allogeneic CD8<sup>+</sup> T cells *in vitro* (Elbe *et al.*, 1994), as well as prime the immune system against transplantation antigens (Lenz *et al.*, 1996) and exogenous hepatitis B (Bohm *et al.*, 1995) *in vivo*. More recently, skin- and bone marrow-derived DCs obtained from MHC class II<sup>-/-</sup> C57BL/6 mice and pulsed with dinitrofluorobenzene (DNFB) induced



thymic DCs express high levels of CD8 but low levels of CD4, some human thymic DCs express low levels of CD8 but high levels of CD4 (Ardavin, 1997; Sotzik *et al.*, 1994).

#### E. PERIPHERAL TOLERANCE

Peripheral selection, dependent on TCR-ligand interactions, differs from thymic selection with regard to specificity and mechanism, requiring binding of antigen to the TCR and induction of T cell clonal expansion. In contrast, tolerance to self-antigens that are restricted to the periphery may occur through the anergy of self-reactive T cells as a consequence of down-regulation of the  $\alpha\beta$  TCR and CD8 (Rocha and von Boehmer, 1991). Protection from myelin basic protein (MBP)-induced encephalitis can be induced by intravenous injection of thymic DCs either pulsed with the immunodominant peptide of MBP or isolated from thymi inoculated *in vivo* with MBP (Khoury *et al.*, 1995). Such a property is not restricted to thymic DCs, however, because intravenous administration of antigen-pulsed LCs or splenic DCs can selectively suppress delayed-type hypersensitivity responses (Morikawa *et al.*, 1992, 1993). As discussed earlier, DCs may be rendered tolerogenic after incubation with IL-10 (Enk *et al.*, 1993a,b) or by exposure to UV irradiation, which induces DNA damage and perturbs the expression of CD80/CD86 (Simon *et al.*, 1991; Vink *et al.*, 1996, 1997). In both cases immature DCs seem more susceptible to exogenous factors that can render them tolerogenic, than are fully mature DCs (Buelens *et al.*, 1997b; Young *et al.*, 1993). Interestingly, the induced tolerance may represent a skewing of the immune response toward the type 2 pathway (Morikawa *et al.*, 1995). Such skewing may also explain (1) the immune privilege of the anterior ocular chamber, where high levels of TGF- $\beta$ 2 may alter the function of local DCs (Streilein, 1997), and (2) oral tolerance induced by low doses of antigens (Weiner, 1997). In contrast, oral tolerance induced by high doses of antigen appears to depend on T cell deletion and anergy.

Two distinct DC populations isolated from mouse spleen and lymph node, CD8 $\alpha^+$  and CD8 $\alpha^-$ , may explain the induction of tolerance versus immunity. Those DCs that bear CD8 $\alpha^-$  express Fas ligand and restrict peripheral CD4 T cell responses by initiating Fas-mediated apoptosis (Lu *et al.*, 1997; Suss and Shortman, 1996), whereas CD8 $\alpha^+$  DCs induce a vigorous proliferative response in CD4 $^+$  T cells. The proliferative response of CD8 T cells is markedly less on stimulation by CD8 $^+$  DC than by conventional CD8 $^-$  DCs, but this reduced proliferation occurs without involving FasL-induced apoptosis, and is completely reversed by the addition of exogenous IL-2 (Kronin *et al.*, 1996).

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## VI. Interactions of Dendritic Cells with B Lymphocytes

### A. AN ABBREVIATED VIEW OF A B LYMPHOCYTE LIFE

Naive B lymphocytes, generated within the bone marrow, migrate into the secondary lymphoid organs where they either die or are recruited into the recirculating B cell pool. Without antigenic encounter, these naive B cells recirculate through the blood, secondary lymphoid organs (tonsils, lymph nodes, spleen, and mucosal-associated lymphoid tissues), lymph, and back to the blood. However, once antigens/pathogens are localized within a secondary lymphoid organ, the recirculating naive B lymphocytes (and naive T lymphocytes) bearing specific antigen receptors are retained.

During T cell-dependent immune responses, naive B cells with specific antigen receptors are activated in association with antigen-specific T cells and interdigitating dendritic cells within the extrafollicular areas. The activated B blasts undergo either terminal differentiation toward plasma cells or become germinal center (GC) founder cells that will migrate into primary follicles or the dark zone of established germinal centers in secondary follicles. The GC founder cells undergo clonal expansion and differentiation into proliferating centroblasts that form and sustain the dark zones. At this level, point mutations are introduced into the immunoglobulin variable (IgV) region genes, in a stepwise fashion. Three types of mutants can be generated, including high-affinity, low-affinity, and autoreactive mutants, which compose the basal light zone of the GC. The survival of these somatic mutants depends on their binding to the low levels of antigen–antibody immune complexes on the surface of follicular dendritic cells (FDCs). High-affinity mutants capture antigen, process it, and present it to GC T cells. Autoreactive mutant clones and low-affinity mutants are deleted. The selected high-affinity centrocytes present processed antigen to antigen-specific T cells, which are induced to express CD40 ligand (CD40L) and secrete cytokines, including IL-4 and IL-10. These are all key elements for B cell survival, proliferation, and isotype switching. This cognate T cell–B cell interaction results in the expansion and isotype switching of high-affinity centrocytes. Finally, the high-affinity isotype-switched centrocytes differentiate into memory B cells in the presence of prolonged CD40L signaling or into plasma cells when CD40L signaling is removed. During secondary humoral immune responses, recirculating memory B cells can be activated in extrafollicular areas, giving rise to plasma cells and GC founder cells. (see also reviews in Liu and Banchereau, 1996b; Kelsoe, 1996; MacLennan, 1994; and *Immunological Reviews* 156 (1997), which is dedicated to the anatomy of antigen-specific immune responses).

## B. FOLLICULAR DENDRITIC CELLS AND GERMINAL CENTER DENDRITIC CELLS

Thirty years ago, the follicular dendritic cell was identified as a new cell type within both primary and secondary follicles, based on its ability to trap antigens in the form of immune complexes on the surface of complicated dendritic processes (Nossal *et al.*, 1968a,b; Said *et al.*, 1997; Szakal and Hanna, 1968). The origin of these FDCs, hematopoietic versus nonhematopoietic, has been a much debated issue. However, it is now clear that there are two very distinct populations: (1) the follicular dendritic cell of mesenchymal origin (fibroblast-like) (Matsumoto *et al.*, 1997) and (2) the germinal center dendritic cell (Grouard *et al.*, 1996) or antigen-transporting cell (Szakal *et al.*, 1989) of hematopoietic origin.

### 1. Follicular Dendritic Cells

Human FDCs display a fibroblast-like morphology together with extensive cytoplasmic extensions and foldings (Fig. 8). FDCs also contain one to several large round nuclei with dispersed chromatin and clear nucleoli. The phenotype of human FDCs is better characterized than that of mouse or rat FDCs (Dijkstra and Van den Berg, 1991; Schriever and Nadler, 1992; Tew *et al.*, 1990). All FDCs express the monocyte marker CD14, the three types of complement receptors (CR1/CD35, CR2/CD21, CR3/CD11b), and the Ig Fc $\gamma$  receptor (CD32). FDCs specifically express the longer form of CD2/CD21 that has 16 short-chain consensus repeats versus the 15 short-chain consensus repeats of B cells (Liu *et al.*, 1997b). A subset of FDCs in the GC light zone expresses the low-affinity receptor for IgE

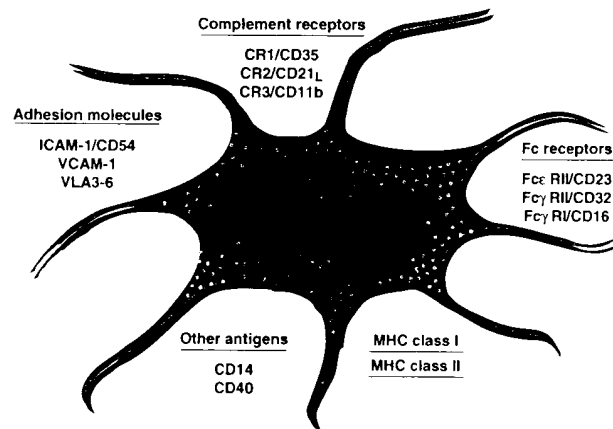
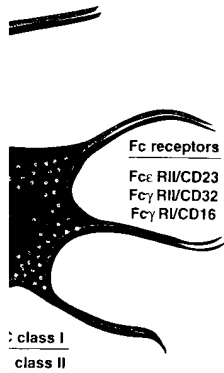


FIG. 8. Phenotype of follicular dendritic cells, showing molecules that they express.

cell was identified as a new cell type, based on its ability to trap antigens on the surface of complicated structures. Said *et al.*, 1997; Szakal and Schrier, 1997). However, it is now clear that the follicular dendritic cell of the spleen (Mason *et al.*, 1997) and (2) the dendritic cell (Said *et al.*, 1996) or antigen-transporting cell (Said *et al.*, 1997).

#### Follicular Dendritic Cells

Morphology together with extensive antigenic characterization (Fig. 8). FDCs also contain one chromatin and clear nucleoli. They are characterized than that of mouse dendritic cells (Said *et al.*, 1991; Schriever and Nadler, 1991). The monocyte marker CD14, CR1/CD35, CR2/CD21, CR3/CD35, and CD40 are expressed by FDCs specifically express the B cell chain consensus repeats versus dendritic cells (Liu *et al.*, 1997b). A subset of FDCs express a low-affinity receptor for IgE



expressing molecules that they express.

(FcεRII, CD23), which also represents one of the ligands for CD21. Thus, these complement receptors and Fc receptors confer FDCs with an efficient mechanism for trapping Ag-Ab-C3 complexes.

FDCs express a wide range of adhesion molecules, including ICAM-1/CD54, VCAM-1, VLA-3, -4, -5, -6, and VLA β chain. Experiments *in vitro* have concluded that adhesion between B cells and FDCs is mediated by the interaction between ICAM-1/CD54 and LFA-1/CD11a, as well as between VCAM-1 and VLA-4 (Freedman *et al.*, 1990; Koopman *et al.*, 1991). Interestingly, purified ICAM-1 molecules appear to deliver survival signals to human GC B cells through LFA-1 molecules, indicating that these types of molecules may perform other functions in addition to adhesion (Koopman *et al.*, 1993). FDCs express CD40, and activated human T cells can induce a human FDC-like cell line to proliferate in a CD40L-dependent fashion (Kim *et al.*, 1994), suggesting that CD40/CD40L interactions may be involved in FDC-T cell interactions *in vivo*.

The expression of Fc receptors by FDCs renders their phenotypic analysis difficult, because of increased background staining. Accordingly, the expression on human FDCs of the B cell markers CD19, CD20, and CD24, the panleukocyte antigen CD45, and class II MHC antigens remains controversial. In the mouse, adoptive transfer of B cells from class II MHC I<sup>E</sup> transgenic mice into congenic mice has suggested that the host FDCs do not synthesize MHC class II antigens but rather capture the donor class II MHC I<sup>E</sup> molecules shed by surrounding donor GC B cells (Gray *et al.*, 1994).

The FDCs organize the primary follicles as evidenced by the lack of FDCs and follicles in TNF knockout mice (Liu and Banchereau, 1996b; Pasparrakis *et al.*, 1996). It also seems that FDCs may enhance the growth and differentiation of activated B cells. Human FDC clusters promote moderate and short-term autologous B cell proliferation. FDCs also mediate a powerful stimulatory effect on the secretion of IgG, IgA, and IgM by CD40-activated B cells, most particularly when cells are cultured with IL-2 and IL-10 (Grouard *et al.*, 1995). There is also evidence that suggests that FDCs inhibit apoptosis in GC B cells by rapid inactivation of preexisting endonuclease, using a mechanism distinct from CD40 ligation (Lindhout *et al.*, 1995). Development of the follicle requires a pre-FDC of mesenchymal origin that expresses TNF-RI (Matsumoto *et al.*, 1997) and B cells that produce lymphotoxin α (LT-α) (Fu *et al.*, 1998; Gonzalez *et al.*, 1998).

#### 2. Germinal Center Dendritic Cells

CD4<sup>+</sup> CD11c<sup>+</sup> germinal center dendritic cells (GCDCs) have been found among CD4<sup>+</sup> CD3<sup>-</sup> cells within the germinal center of human

tonsils. They represent less than 0.5–1% of GC cells and are distributed in both the dark and light zones. These GCDCs express all Fc $\gamma$  receptors (CD16, CD32, CD64) as well as the three complement receptors (CR1, CR2, CR3), accounting for their efficient binding of immune complexes. Isolated GCDCs display (1) poor uptake of soluble FITC-dextran or phagocytosis of FITC-latex beads, (2) potent induction of allogeneic naive CD4<sup>+</sup> T cell proliferation, and (3) a strong capacity to enhance B cell growth and differentiation (Dubois and Briere, personal communication). The current hypothesis is that these GCDCs represent the mature form of the CD11c<sup>+</sup> precursors in circulating blood (O'Doherty *et al.*, 1993; Thomas *et al.*, 1993) and may correspond to the interstitial DCs generated *in vitro* from CD34<sup>+</sup> HPCs. These GCDCs also likely correspond to the previously described antigen-transporting cells (Szakal *et al.*, 1985), which coordinate the generation of memory T and B cells that share specificity for a given antigen.

### C. DENDRITIC CELL AND B CELL DIALOGUES

Several *in vitro* and *in vivo* observations have suggested the importance of DCs in the establishment of humoral responses (Cebra *et al.*, 1994; Flamand *et al.*, 1994; Francotte and Urbain, 1985; Inaba *et al.*, 1983a; Inaba and Steinman, 1985; Schrader *et al.*, 1990; Sornasse *et al.*, 1992; Spalding and Griffin, 1986), but it is a common understanding that DCs act to select and activate antigen-specific resting T cells that subsequently induce B cell responses. More specifically, on priming by DCs, activated T cells express CD40 ligand (CD40L), which in turn interacts with CD40-expressing B cells to form a cellular triad. Activated T cells promote B cell survival (Liu, 1989), proliferation (Banchereau *et al.*, 1991), differentiation, and isotype switching (Defrance *et al.*, 1992; Jabara *et al.*, 1990; Malisan *et al.*, 1996) through cytokines CD40 and CD70 (Jacquot *et al.*, 1997). However, there is now evidence that DCs directly interact with B cells to regulate humoral responses.

#### 1. Dendritic Cells and Humoral Responses *in Vivo*

In murine models, the requirement for splenic adherent cells in primary antibody synthesis (Mosier, 1967) led to the discovery of a key role played by DCs in such responses (Inaba *et al.*, 1983a). Using hapten-carrier conjugates, DCs can sensitize carrier-specific T cells, which in turn interact with hapten-specific B cells that then proliferate and differentiate (Inaba and Steinman, 1985). The *in vivo* T cell priming obtained by administration of antigen-pulsed DCs (Inaba *et al.*, 1990a,b) is followed by the appearance of antigen-specific immunoglobulin in serum (Berg *et al.*, 1994; Flamand *et al.*, 1994; Francotte and Urbain, 1985; Sornasse *et al.*, 1992). Ig levels

DC cells and are distributed. DCs express all Fc $\gamma$  receptors and complement receptors (CRI, binding of immune complexes, and soluble FITC-dextran or induction of allogeneic naive capacity to enhance B cell response, personal communication). They represent the mature form of DC (O'Doherty *et al.*, 1993; these interstitial DCs generated also likely correspond to the DCs (Szakal *et al.*, 1985), which interact with B cells that share specificity.

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re suggested the importance of these responses (Cebra *et al.*, 1994; Cebra, 1985; Inaba *et al.*, 1983a; Inaba *et al.*, 1990; Sornasse *et al.*, 1992; Inaba, 1992). Upon understanding that DCs prime T cells that subsequently interact with B cells, priming by DCs, activated T cells in turn interacts with CD40-expressing B cells. Activated T cells promote B cell responses (Cebra *et al.*, 1994; Cebra, 1985; Inaba *et al.*, 1983a; Inaba *et al.*, 1990; Sornasse *et al.*, 1992; Inaba, 1992), differentiation (Cebra *et al.*, 1994; Inaba *et al.*, 1990; Inaba, 1992) and CD70 (Jacquot *et al.*, 1992). DCs directly interact with B

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become detectable after a second challenge with soluble antigen, a few days after DC injection (Liu and MacPherson, 1993; Sornasse *et al.*, 1992). Such studies unfortunately cannot confirm the presumed direct interaction of DCs with B cells. Immunohistochemical studies have, however, shown that human tonsillar interdigitating dendritic cells from T cell areas colocalize with naive B cells (Bjorck *et al.*, 1997b). Furthermore, DC-B cell clusters have been observed *in vivo* in rat lymph (Kushnir *et al.*, 1998).

## 2. Dendritic Cells and Humoral Responses in Vitro

To analyze the possible interactions between DCs and B cells in a T cell-dependent context, *in vitro*-generated DCs have been cocultured with allogeneic B cells using a CD40 ligand-transfected cell line with or without cytokines (Dubois *et al.*, 1997). Such a CD40L-expressing cell line can serve as an experimental surrogate of activated T cells.

a. *Dendritic Cells Enhance the Proliferation of Activated B Cells.*

Through the release of uncharacterized soluble factors (different from sgp80, IL-12, and IL-10), *in vitro*-generated DCs increase the yield of viable human naive and memory B cells activated solely through their CD40 antigen. The proliferation of B cells activated with particles of *Staphylococcus aureus* Cowan I (Banchereau and Rousset, 1992) is also enhanced by DCs. DCs can further enhance the considerable proliferation of CD40-activated B cells that occurs in response to IL-4, IL-13, and IL-10. Furthermore, DCs allow CD40-activated B cells to proliferate in response to IL-2 (Dubois *et al.*, 1997; Fayette *et al.*, 1997). The induction by DCs of IL-2-mediated B cell proliferation necessitates CD40 activation of DCs and involves both IL-12 and sgp80 (Dubois, 1998).

#### b. Dendritic Cells Induce B Cell Differentiation.

1. Naive B cells can secrete IgM in response to IL-2. Addition of DCs allows CD40-activated naive B cells to produce IgM in response to IL-2. IL-12 represents the critical DC-derived molecule, secreted following CD40 engagement, that permits the differentiation of naive B cells into IgM-secreting plasma cells (Arpin *et al.*, 1997; Dubois, 1998). This further establishes the underestimated role of IL-12 on B cells, previously shown to enhance (1) the proliferation and polyclonal Ig secretion of BCR-activated human peripheral blood B cells cultured in the presence of IL-2 (Jelinek and Braaten, 1995) and (2) the antigen-specific antibody response by peripheral blood mononuclear cells (Clerici *et al.*, 1993; Luzzati *et al.*, 1997; Uherova *et al.*, 1996). Furthermore, the primary humoral response *in vivo* to a microbial antigen in SCID mice engrafted with human PBLs was shown to be IL-12-dependent (Westerink *et al.*, 1997). IL-12-treated

mice respond to proteins and haptens with increased IgG<sub>2a</sub> and decreased IgG<sub>1</sub> antibodies through mechanisms that are both dependent and independent of IFN- $\gamma$  (Buchanan *et al.*, 1995; Germann *et al.*, 1995; McKnight *et al.*, 1994; Metzger *et al.*, 1996). Thus, in addition to priming T cells toward Th1 development, DC-secreted IL-12 may directly signal naive B cells during the initiation of the immune response.

2. *Memory B cells differentiate without exogenous cytokines.* In the absence of exogenous cytokines, DCs potentiate the differentiation of CD40-activated memory B cells into IgG- and IgA-secreting cells (Dubois *et al.*, 1997). Although the effect is IL-12 independent, endogenous IL-6 represents the major factor responsible for the observed differentiation (Dubois, 1998). This is consistent with the critical role of IL-6 in B cell differentiation (Burdin *et al.*, 1996; Kishimoto, 1985; Kishimoto *et al.*, 1984). DCs also secrete soluble IL-6R $\alpha$  chain (sgp80), which allows the formation IL-6/sgp80 complexes that bind with high affinity to the IL-6R transducing chain, gp130 (Peters *et al.*, 1997), thus resulting in enhanced IL-6 action.

3. *Skewing of isotype switching toward IgA<sub>1</sub> and IgA<sub>2</sub>.* Provided that naive B cells are activated through CD40, DCs induce isotype switching toward IgA in the absence of exogenous cytokines (Fayette *et al.*, 1997). Induction of surface IgA-expressing B cells is quantitatively comparable to that obtained with the combination of IL-10 and TGF- $\beta$  (Defrance *et al.*, 1992). The DC-induced expression of sIgA<sup>+</sup> B cells is partially mediated by TGF- $\beta$  (Fayette *et al.*, 1997). Although DCs allow CD40-activated naive B cells to express surface IgA, IL-10 is necessary for their differentiation into IgA-secreting cells. In the presence of IL-10 and TGF- $\beta$ , naive B cells secrete both IgA<sub>1</sub> and IgA<sub>2</sub> subclasses (Fayette *et al.*, 1997). These observations extend earlier studies with mouse B cells (Cebra *et al.*, 1994; Schrader and Cebra, 1993; Schrader *et al.*, 1990) and pre-B cell lines (Spalding and Griffin, 1986), which were shown to secrete high levels of IgA in the presence of a combination of polyclonally activated T cells or Th2 clones and DCs. Thus, it is tempting to speculate that DCs generated *in vitro* possibly share an important role in the regulation of mucosal humoral responses with mucosal DCs (Kelsall *et al.*, 1996). Studies in rats have also shown that DCs can skew the antibody responses toward the Th2 type (Wykes *et al.*, 1998). This study further indicates that DCs can capture and retain unprocessed antigen *in vitro* and *in vivo* and transfer it to naive B cells.

4. *Distinct subpopulations of dendritic cells differentially regulate B cell responses.* DCs and monocytes display a comparable ability to enhance CD40-activated B cell proliferation, whereas DCs are more efficient than monocytes in inducing memory B cells to secrete IgG and IgA in the absence of cytokines (Dubois *et al.*, 1997). DCs derived from either CD34<sup>+</sup>

increased IgG<sub>2a</sub> and decreased re both dependent and independent. Hermann *et al.*, 1995; McKnight in addition to priming T cells IL-12 may directly signal naive B response.

*exogenous cytokines.* In the otentiate the differentiation of and IgA-secreting cells (Dubois independent, endogenous IL-6 or the observed differentiation e critical role of IL-6 in B cell imoto, 1985; Kishimoto *et al.*, chain (sgp80), which allows the with high affinity to the IL-6R (97), thus resulting in enhanced

*IgA<sub>1</sub> and IgA<sub>2</sub>.* Provided that , DCs induce isotype switching cytokines (Fayette *et al.*, 1997). ILs is quantitatively comparable IL-10 and TGF- $\beta$  (Defrance *et al.* IgA<sup>+</sup> B cells is partially mediated gh DCs allow CD40-activated s necessary for their differentiation of IL-10 and TGF- $\beta$ , naive es (Fayette *et al.*, 1997). These ouse B cells (Cebra *et al.*, 1994; *al.*, 1990) and pre-B cell lines shown to secrete high levels of polyclonally activated T cells or o speculate that DCs generated in the regulation of mucosal sall *et al.*, 1996). Studies in rats antibody responses toward the further indicates that DCs can *vitro* and *in vivo* and transfer

*cells differentially regulate B cell* comparable ability to enhance as DCs are more efficient than o secrete IgG and IgA in the DCs derived from either CD34<sup>+</sup>

hematopoietic progenitors or monocytes, but not monocytes themselves, induce surface IgA expression on CD40-activated naive B cells in the absence of cytokines. Both the interstitial DCs and LCs are able to enhance the proliferation of CD40-activated B cells and to induce the differentiation of memory B cells, but only interstitial DCs can induce naive B cells to differentiate into IgM-secreting cells in response to CD40 ligation and IL-2 (Caux *et al.*, 1997). This suggests that dermal DCs rather than epidermal LCs could be critical in launching primary B cell responses.

## VII. Dendritic Cells in Clinical Disease States

### A. AUTOIMMUNITY

#### 1. Rheumatoid Arthritis

Synovial fluid contains cells that are comparable in function, phenotype, and structure to blood DCs, although the frequency (1–5%) is 10-fold greater. The reason for DC accumulation in the articular cavity is unknown, as is any role of DCs perpetuating the joint inflammation characteristic of this disease (Thomas *et al.*, 1994; Zvaifler *et al.*, 1985). Phenotypic analyses suggest that synovial DCs are not fully activated, however, because they express low levels of CD80 and CD86. Fluids from affected joints also contain modulators of DC maturation, e.g., IL-10 (Summers *et al.*, 1995a,b, 1996).

#### 2. Psoriasis

Local activation of T lymphocytes is regarded as an important immunological component of psoriatic skin lesions. Within psoriatic plaques large numbers of dermal (interstitial) DCs are surrounded by T cells (Nestle *et al.*, 1994). Psoriatic DCs are more active stimulators of autologous T cell proliferation than are either psoriatic blood-derived or normal skin-derived DCs. These psoriatic DCs are not more potent in supporting superantigen-induced T cell proliferation, however, which suggests that the autostimulatory potency of psoriatic skin DCs may be a critical alteration leading to the skin lesion (Nestle *et al.*, 1994). In contrast to normal skin DCs, psoriatic DCs express high levels of CD1b and CD1c. Whether this represents a marker of the activation status of psoriatic DCs or an explanation for the enhanced autostimulatory capacity (Fivenson and Nickoloff, 1995) remains to be established.

### B. TRANSPLANTATION

#### 1. Dendritic Cells and Transplantation Immunity

Interstitial DCs were originally suspected to be the passenger leukocytes that led to the primary allograft reaction (Hart, 1997; Hart and Fabre,

1981; Hart *et al.*, 1981). Indeed, DCs have been shown to migrate from cardiac (Larsen *et al.*, 1990b) or liver allografts (Qian *et al.*, 1994) to the T cell areas of recipient spleens, where they effectively prime antigen-specific immune responses. The depletion of DCs from solid organ grafts such as kidney (McKenzie *et al.*, 1984), heart (McKenzie *et al.*, 1984), Langerhans islets (Faustman *et al.*, 1984), and thyroid (Iwai *et al.*, 1989) prolongs graft survival. Clinical trials aimed at depleting donor kidney DCs have also shown some beneficial effects (Brewer *et al.*, 1989). Furthermore, MHC-incompatible tissue devoid of DCs only provokes responses comparable to those induced by minor histocompatibility differences (Lechler and Batchelor, 1982a,b)). Very little is known about the role of DCs in graft-versus-host disease, but they likely play a role because all the involved sites are populated by DCs. DCs, which are radioresistant, theoretically contribute to direct donor T lymphocyte allosensitization and prime for the donor immune reactivity that results in the clinical syndrome of graft-versus-host disease.

## 2. Dendritic Cells and Transplantation Tolerance

The spontaneous acceptance of transplanted livers in mice despite MHC mismatch suggests the existence of tolerance induction pathways that can be exploited especially by this organ (Qian *et al.*, 1994). Inasmuch as liver represents an early site of hematopoiesis, it has been hypothesized that DC precursors are seeded from the liver graft to recipient lymphoid tissue after transplantation. Supporting evidence derives from the identification of donor-derived cells in recipient bone marrow, or spleen, whereas such cells are not observed in marrows of mice rejecting heart allografts (Lu *et al.*, 1995b). Microchimerism has also been detected in the tissues or blood of human kidney or liver transplants studied 2 to 30 years postoperatively (Starzl *et al.*, 1992, 1993). Some of the donor cells appear to have been candidate DCs. Although it can be argued that this microchimerism is merely a consequence of long-term allografting (Starzl *et al.*, 1997; Thomson *et al.*, 1995), it is equally plausible that microchimerism actively supports induction of transplantation tolerance (Starzl *et al.*, 1996). For example, costimulatory molecule-deficient DC progenitors (class II MHC, B7.1<sup>dim</sup>, B7.2<sup>-</sup>) grown in low concentrations of GM-CSF alone fail to stimulate a primary MLR and induce donor-specific T cell anergy (Lu *et al.*, 1995a). Administering costimulatory molecule-deficient DC precursors to normal mice also allows the subsequent engraftment of vascularized cardiac allografts (Fu *et al.*, 1996, 1997). Thus in addition to having a role in central tolerance, DCs are now regarded as potential modulators of peripheral immune responses, offering a new approach to the immunosup-

been shown to migrate from grafts (Qian *et al.*, 1994) to the kidney effectively prime antigen-presenting DCs from solid organ grafts in heart (McKenzie *et al.*, 1984), and thyroid (Iwai *et al.*, 1989) and depleting donor kidney DCs (Ver *et al.*, 1989). Furthermore, it provokes responses compatible with differences (Lechler *et al.*, 1994) about the role of DCs in a role because all the involved are radioresistant, theoretically desensitization and prime for the clinical syndrome of graft-

#### Intestinal Tolerance

and livers in mice despite MHC induction pathways that can (Lu *et al.*, 1994). Inasmuch as liver has been hypothesized that it to recipient lymphoid tissue derives from the identification of marrow, or spleen, whereas such rejecting heart allografts (Lu *et al.*, 1994) detected in the tissues or blood 2 to 30 years postoperatively or cells appear to have been that this microchimerism is long (Starzl *et al.*, 1997; Thomsen *et al.*, 1996). For example, microchimerism actively suppresses (Starzl *et al.*, 1996). For example, progenitors (class II MHC, is of GM-CSF alone fail to antigen-specific T cell anergy (Lu *et al.*, 1994). Culture-deficient DC precursors engraftment of vascularized is in addition to having a role as potential modulators of approach to the immunosup-

pressive therapy of allograft rejection or autoimmunity (Stephens and Thomson, 1996).

#### C. CONTACT ALLERGY

Contact sensitivity (CS) is a T cell-mediated immune reaction occurring after cutaneous immunization and challenge with low molecular weight chemicals (haptens) that covalently bind to self- or exogenous proteins. Hapten-modified proteins are then processed by APCs (Langerhans cells) that subsequently migrate to draining lymph nodes to initiate immune responses (Girolomoni *et al.*, 1995; Macatonia *et al.*, 1986, 1987; Sullivan *et al.*, 1985; Toews *et al.*, 1980). Unlike classical delayed-type hypersensitivity (DTH) to proteins or cellular antigens, mediated primarily by MHC class II-restricted CD4<sup>+</sup> T cells (Cher and Mosmann, 1987), the T cell response to haptens appears more complex and may involve CD4<sup>+</sup> T cells and/or CD8<sup>+</sup> T cells, depending on the hapten and the mouse strain (Grabbe and Schwarz, 1998). Responses to dinitrofluorobenzene in C57BL/6 mice are mediated by MHC class I-restricted CD8<sup>+</sup> effector T cells that can be primed by class I MHC<sup>+</sup>, class II MHC<sup>-</sup> DCs. The response is down-regulated by CD4 regulatory T cells that are primed by class II MHC<sup>+</sup>, class I MHC<sup>+</sup> (Bour *et al.*, 1995; Krasteva *et al.*, 1998).

IL-10 is released during the induction phase of contact sensitivity and was shown in prior functional studies to convert LCs from potent inducers of primary immune responses specifically to tolerizing cells *in vitro*. Data indicate that *in vivo* application of IL-10 before allergen exposure induces antigen-specific tolerance in mice and that IL-10 might act via inhibition of proinflammatory cytokines (Enk *et al.*, 1994).

#### D. DENDRITIC CELLS AS IMPORTANT APCs IN ASTHMA

IgE plays an important role in asthma, with total serum IgE levels closely related to both clinical expression of the disease and airway hyperresponsiveness. IgE binds to a high-affinity cell surface receptor (FcεRI), which is present not only on mast cells but also on cutaneous DCs (Maurer *et al.*, 1996; Stingl and Maurer, 1997; Stingl *et al.*, 1977) and, by extension, on DCs of the airway epithelium especially in asthmatics (Semper and Hartley, 1996; Tunon-De-Lara *et al.*, 1996). T lymphocytes, secreting Th2 cytokines such as IL-4 and IL-5 in response to inhaled antigen, play a major role in the pathogenesis of allergic bronchial asthma (Robinson *et al.*, 1992). The network of airway DCs in the lung is particularly well developed to capture inhaled Ag (Gong *et al.*, 1992; Holt *et al.*, 1990; Schon-Hegrad *et al.*, 1991). On encountering inhaled Ag, airway DCs migrate to the draining lymph nodes of the lung and induce primary immune responses (Havenith *et al.*, 1993; Masten *et al.*, 1997; Xia *et al.*,

1995). DCs are also important for presenting inhaled Ag to previously primed Th2 lymphocytes in the lung, leading to chronic eosinophilic airway inflammation (Lambrecht *et al.*, 1998). In contrast, B cells do not play an important role in the induction of airway inflammation (Kosgren, 1997), and alveolar macrophages appear to suppress the activity of other APCs (Holt *et al.*, 1985; Thepen *et al.*, 1992).

The number of DCs is significantly higher in the airways of asthmatics compared with control subjects, as is the proportion of DCs expressing Fc $\epsilon$ RI- $\alpha$  (Semper and Hartley, 1996; Tunon-De-Lara *et al.*, 1996). Thus DCs may play a significant role in the onset and perpetuation of allergic asthma, and targeting DCs may represent an important new approach to the treatment of asthma. Indeed the therapeutic benefit of steroids in this disease may be due to an alteration of DC functions (Nelson *et al.*, 1995).

#### E. DENDRITIC CELLS AND BACTERIA

Immature DCs phagocytose dead and live bacteria, including Calmette-Guerin organisms, the attenuated strain of *Mycobacterium bovis* used as a vaccine against tuberculosis (Inaba *et al.*, 1993), *Mycobacterium tuberculosis* (Larsson *et al.*, 1997), *Chlamydia trachomatis* (the agent that results in blindness) (Larsson *et al.*, 1997), *Salmonella typhimurium* (Svensson *et al.*, 1997), *Listeria monocytogenes* (MacLean *et al.*, 1996), *Escherichia coli* (Eloranta *et al.*, 1997; Svensson *et al.*, 1997), *Bordetella bronchiseptica* (Guzman *et al.*, 1994a,b), and *Borrelia burgdorferi*, the agent of Lyme disease (Filgueira *et al.*, 1996). *Listeria* is able to kill DCs, possibly through the production of listeriolysin. DCs process the live bacteria for peptide presentation by class I and class II MHC molecules. Bacterial infections of DCs result in their activation as demonstrated by the increased expression of surface costimulatory molecules (CD54, CD40, CD80, CD83, CD86) and the secretion of multiple cytokines, including TNF, IL-1, IL-12, and IFN- $\alpha$  and IFN- $\beta$  (Thurnher *et al.*, 1997). Bacterial-induced maturation is in turn associated with a decreased antigen capture capacity.

#### F. DENDRITIC CELLS AND PARASITES

Human infections with *Leishmania* parasites range from self-healing cutaneous to uncontrolled, diffuse cutaneous disease, and from subclinical to fatal visceral disease. Immature DCs can phagocytose the organism *in vitro*, and LCs infected by *Leishmania major* are present in the dermal infiltrate of lesional skin (Blank *et al.*, 1993). DCs restrain intracellular parasite replication through uncharacterized mechanisms (Moll *et al.*, 1993). *Leishmania*-infected LCs can migrate into the draining lymph nodes, where they mature and activate resting and memory T cells with specificity for *Leishmania*. Macrophages are unable to elicit primary responses and

ing inhaled Ag to previously to chronic eosinophilic airway contrast, B cells do not play an inflammation (Kosgren, 1997), as the activity of other APCs

in the airways of asthmatics proportion of DCs expressing i-De-Lara *et al.*, 1996). Thus and perpetuation of allergic a important new approach to utic benefit of steroids in this ctions (Nelson *et al.*, 1995).

bacteria, including Calmette-*Mycobacterium bovis* used as 93), *Mycobacterium tubercu-omatis* (the agent that results *la typhimurium* (Svensson *et al.*, 1996), *Escherichia coli* 7), *Bordetella bronchoseptica gdorferi*, the agent of Lyme to kill DCs, possibly through the live bacteria for peptide olecules. Bacterial infections ated by the increased expres- D54, CD40, CD80, CD83, kines, including TNF, IL-1, *al.*, 1997). Bacterial-induced sed antigen capture capacity.

ites range from self-healing disease, and from subclinical phagocytose the organism *in or* are present in the dermal ). DCs restrain intracellular d mechanisms (Moll *et al.*, nto the draining lymph nodes. emory T cells with specificity elicit primary responses and

are poorly efficient in stimulating secondary responses (Moll *et al.*, 1995; Will *et al.*, 1992). Lymph node DCs carry persistent parasites that may result in the sustained stimulation of memory T cells and allow the maintenance of protective immunity. After intravenous administration of *Leishmania donovani* (the species responsible for visceral leishmaniasis), the parasites are found within the marginal metallophil macrophages, where they are degraded. However, a small proportion of the parasites localizes to DCs within the periarteriolar lymphocytic sheath, where they persist and stimulate production of IL-12 (Gorak *et al.*, 1998).

In humans, infections with *Toxoplasma gondii* are largely asymptomatic, although fetal contamination results in malformations that can be extremely severe. Moreover, life-threatening systemic toxoplasmosis can occur in AIDS patients and other conditions associated with profound immune suppression. *Toxoplasma* antigens induce the redistribution of DCs to T cell areas and activate the secretion of IL-12 by DCs but not by macrophages (Sousa *et al.*, 1997). It remains to be determined whether the *Toxoplasma* parasites that invade the gut are directly taken up by DCs or whether macrophages capture and process them (Johnson and Sayles, 1997). *Toxoplasma* has been shown to infect human DCs (T. Curiel, personal communication).

#### G. DENDRITIC CELLS AND VIRUSES

The role of DCs as potentiators/initiators of antiviral immune responses has been well documented in murine systems. In particular, DCs are the most efficient APCs in stimulating recall CTL responses against Sendai viruses (Kast *et al.*, 1990), Herpes simplex virus (Hengel *et al.*, 1987), and influenza virus (Nonacs *et al.*, 1992). However, viruses still survive and replicate despite the pressures exerted by the immune response and most particularly by CTLs (Koup, 1994). These include reduced expression of critical antigenic epitopes, genetic variation of MHC class I-restricted CTL epitopes, clonal exhaustion of CTLs, down-regulation of class I MHC-peptide complex expression, production of "immunosuppressive" cytokines such as IL-10, and down-regulation of critical cytokines such as IL-12. DCs represent a cellular target of choice for viruses for multiple reasons. Because of the critical role of DCs in initiating immune reactions, it is very advantageous for the viruses to affect DC viability and biological functions. Furthermore, because of the distribution of DCs throughout body surfaces such as skin and mucosae, DCs provide a means of accessing other cells, such as T cells. Finally, sequestration within the DCs may provide a very efficient strategy for viruses not to be identified by the immune system. As summarized below, evidence is now accumulating that

viruses target DCs for their own benefit, thus antagonizing the function of DCs as initiators and potentiators of antiviral immune responses.

### 1. *Herpesvirus*

Since the discovery of Kaposi's sarcoma-associated herpesvirus, or Herpesvirus-8 (KSHV or HHV8) (Chang *et al.*, 1994), this virus has been shown to be associated with human diseases, including Kaposi's sarcoma (Chang *et al.*, 1994), systemic Castleman's disease (Cesarman *et al.*, 1995), and primary effusion or body-cavity-based lymphoma (Cesarman *et al.*, 1995; Gao *et al.*, 1996). The virus has been localized to malignant cells, although its role in disease pathogenesis remains controversial. HHV8 DNA as well as viral IL-6 RNA transcripts have been detected in CD83<sup>+</sup>, fascin/p55<sup>+</sup>, CD68<sup>+</sup> cells from cultured bone marrow stromal cells in 15 out of 15 myeloma patients and from 2 out of 8 patients with monoclonal gammopathy of undetermined significance, a precursor to myeloma (Rettig *et al.*, 1997). This initial finding sparked controversy because results could not be reproduced by several groups (Cottoni and Uccini, 1997; Masood *et al.*, 1997; Parravicini *et al.*, 1997; Whitby *et al.*, 1997; Yi *et al.*, 1998), but were corroborated by other investigators (Brouss *et al.*, 1997). HHV8 has been demonstrated by *in situ* hybridization within the bone marrow of myeloma patients (Said *et al.*, 1997). It has been proposed that HHV8 or KSHV, if it is actually present in DCs, may stimulate and maintain abnormal plasma cell proliferation in myeloma through alterations in the bone marrow microenvironment and production of viral IL-6 (vIL-6). Nevertheless, the initial genetic alterations that lead to plasma cell transformation remain to be identified.

### 2. *Cytomegalovirus*

Cytomegalovirus (CMV) is a ubiquitous pathogen that is a major cause of morbidity and mortality in immunocompromised individuals, including patients with AIDS or those who have undergone bone marrow or solid organ transplantation (Britt and Mach, 1996). CMV is also associated with the development of chronic rejection in organ transplant patients (Grattan *et al.*, 1989; Melnick *et al.*, 1995), and chronic graft-versus-host disease in bone marrow transplant recipients (Lonnqvist *et al.*, 1984; Soderberg *et al.*, 1996). Similar to other herpesviruses, CMV establishes lifelong latency in the host after primary infection, which is characterized by persistence of the viral genome without production of infectious virus. However, transmission of latent CMV can occur through blood transfusion and allografts of bone marrow or solid organs. In long-term cultures of allogeneically stimulated, adherent, monocyte-derived macrophages, human CMV reactivates (Soderberg-Naucler *et al.*, 1997). CD33<sup>+</sup> progenitors of dendritic

thus antagonizing the function of viral immune responses.

Human-associated herpesvirus, or HHV8 (1994), this virus has been associated with Kaposi's sarcoma and B-cell lymphoma (Cesarman *et al.*, 1995). HHV8 is localized to malignant cells, but its role remains controversial. HHV8 has been detected in CD83<sup>+</sup> dendritic cells, bone marrow stromal cells in 15 of 8 patients with monoclonal B-cell precursor to myeloma (Rettig *et al.*, 1997; Masood *et al.*, 1997; Yi *et al.*, 1998), and in B cells (Brouss *et al.*, 1997). HHV8 infection within the bone marrow has been proposed that HHV8 may stimulate and maintain B-cell plasma cell transformation through alterations in the production of viral IL-6 (vIL-6), which may lead to plasma cell transformation.

Measles virus is a major cause of immunosuppression in immunocompromised individuals, including those with transplanted bone marrow or solid organs. CMV is also associated with immunosuppression in transplant patients (Grattan *et al.*, 1984; Soderberg *et al.*, 1994). CMV establishes lifelong latency and is characterized by persistence of infectious virus. However, transfusion and allografts from cultures of allogeneically derived macrophages, human CMV-reactive CD33<sup>+</sup> progenitors of dendritic

cells and monocyte-macrophages are also important reservoirs of latent CMV, whereas T cells, B cells, and CD33<sup>+</sup> mature granulocytes are not (Hahn *et al.*, 1998; Kondo *et al.*, 1994, 1996).

### 3. Dendritic Cells and Influenza Virus

Virtually all DCs are infected on exposure to influenza virus, as demonstrated by expression of the viral proteins hemagglutinin and nonstructural protein 1 (Bhardwaj *et al.*, 1994; Ridge *et al.*, 1998). Infected cells remain viable for more than 2 days, however, and produce little infectious virus. This contrasts with macrophages, which produce infectious virus while undergoing apoptosis (Fesq *et al.*, 1994; Hofmann *et al.*, 1997). Infected DCs, but not infected macrophages or B cells, can induce recall CTL responses by CD8<sup>+</sup> T cells without an absolute requirement for CD4<sup>+</sup> T cell help (Bhardwaj *et al.*, 1994). Several experimental findings account for this specialized function of DCs. First of all, very few infectious virus particles and very small numbers of DCs stimulate a powerful CTL response, as is true of other T cell responses elicited by DCs. DCs also stimulate strong CTL responses after infection with influenza virus inactivated by heat or UV radiation, which almost completely abrogates active viral protein synthesis but apparently maintains viral binding and access to the DC cytoplasm (Bender *et al.*, 1996). Perhaps most relevant to *in vivo* biology, DCs can acquire influenza antigens from virus-infected apoptotic cells and subsequently stimulate MHC class I-restricted CD8<sup>+</sup> CTLs (Albert *et al.*, 1998). This may explain the phenomenon of cross-priming, whereby donor cell antigens are presented by host bystander cells (Bevan, 1977; Fossum and Rolstad, 1986; Huang *et al.*, 1994), as well as the induction of tolerance to tissue-restricted self-antigens (Kurts *et al.*, 1996, 1997a,b).

### 4. Measles Virus

*a. Immunosuppression in Measles.* Measles virus causes a profound immunosuppression that is responsible for the high morbidity and mortality induced by secondary infections (Oldstone, 1996). The mechanism of immune suppression is poorly understood, but it is widely accepted to be the consequence of virus replication within leukocytes, especially within the lymphoid system (Griffin, 1995; Griffin *et al.*, 1994). Infected T cells and monocytes die by apoptosis, particularly within syncytia (Esolen *et al.*, 1995) identifiable *in vivo* in the submucosal areas of tonsils and pharynx (Warthin, 1931) once viral replication has begun, after virus has started replicating. Marked and prolonged alterations of cell-mediated immunity have been noted as a consequence of measles virus infection: T lymphocytopenia, inhibition of delayed-type hypersensitivity responses, and suppres-

sion of antibody responses (McChesney *et al.*, 1986) despite a skewing of T cell responses toward the Th2 pathway (Ward and Griffin, 1993). Interestingly, cutaneous anergy is also observed in response to measles vaccines (Starr, 1964). Three recent studies have highlighted the pathogenic effects of measles virus on human DCs (Fugier-Vivier *et al.*, 1997; Grosjean *et al.*, 1997; Schnorr *et al.*, 1997).

*b. Measles Virus Replicates in DCs.* Wild-type measles virus as well as the Edmonston and Halle vaccine strains can infect human DCs isolated from skin (Langerhans cells) or blood or generated *in vitro* by culturing either CD34<sup>+</sup> HPCs with GM-CSF + TNF (DC<sub>mat</sub>) or blood monocytes with GM-CSF + IL-4 (DC<sub>imm</sub>). This infection results in the surface expression of hemagglutinin on a large proportion of DCs and the generation of giant syncytia. Infectious virions are produced, and DCs eventually undergo apoptosis. The production of virions by DC<sub>imm</sub> is enhanced following contact with T cells in a CD40-dependent fashion as observed with HIV (Pinchuk *et al.*, 1994).

*c. Measles Virus Interferes with Dendritic Cell Stimulation of T Cells.* DCs infected by measles virus show reduced IL-12 production (Fugier-Vivier *et al.*, 1997), as previously reported for monocytes (Karp *et al.*, 1996), and are unable to stimulate proliferation by alloreactive T cells (Fugier-Vivier *et al.*, 1997). Measles virus-infected DCs can also block the allostimulatory capacity of uninfected DCs, even when the infected cells are present at very low numbers (Grosjean *et al.*, 1997). This inhibitory effect is in part due to the release of viable viral particles. However, addition of UV-treated, paraformaldehyde-fixed measles virus-infected DCs also inhibits the allogeneic DC-T cell MLR (Grosjean *et al.*, 1997), supporting an active virus-independent immunosuppression, the mechanisms for which remain to be determined.

The intense immunosuppression induced by measles virus can be explained by a major cytopathic effect on DCs. It is therefore unclear how immunity against measles is ever established. One possibility is that unaffected DCs may acquire measles virus-induced apoptotic bodies, as occurs with influenza (Albert *et al.*, 1998), and subsequently initiate CTL responses. Alternatively, measles virus may differentially affect the various DC subsets or maturational stages, as evidenced by the fact that measles virus-infected immature DCs induce T cell death, whereas T cell viability is not altered by infected mature DCs.

### 5. Dendritic Cells and Retroviruses

The interaction of retroviruses with DCs is best exemplified by human immunodeficiency virus (HIV), the causative agent of AIDS (Fauci, 1996;

*et al.*, 1986) despite a skewing way (Ward and Griffin, 1993). Observed in response to measles viruses have highlighted the pathologic DCs (Fugier-Vivier *et al.*, 1997; ).

Wild-type measles virus as well as can infect human DCs isolated generated *in vitro* by culturing V<sub>F</sub> (DC<sub>mat</sub>) or blood monocytes. Infection results in the surface expression of DCs and the generation of dendritic, and DCs eventually undergo maturation enhanced following contact as observed with HIV (Pinchuk

*Antigen Presenting Cell Stimulation of T Cells.* Induced IL-12 production (Fugier-Vivier *et al.*, 1997) for monocytes (Karp *et al.*, 1997). Infection by alloreactive T cells of infected DCs can also block the maturation of DCs, even when the infected cells are mature (*et al.*, 1997). This inhibitory effect is mediated by viral particles. However, addition of measles virus-infected DCs also suppresses IL-12 production (Fugier-Vivier *et al.*, 1997), supporting the hypothesis that, under certain conditions, the mechanisms for

induced by measles virus can be exploited by DCs. It is therefore unclear how this is mediated. One possibility is that uninfected apoptotic bodies, as occurs with infected cells, subsequently initiate CTLs and differentially affect the various functions of DCs. This is supported by the fact that measles virus induces DC death, whereas T cell viability

#### *Retroviruses*

This is best exemplified by human immunodeficiency virus (HIV), the major agent of AIDS (Fauci, 1996;

Wyatt and Sodroski, 1998). Interestingly, HIV interacts with both the mesenchymal FDCs and the DCs of hematopoietic origin.

*a. Follicular Dendritic Cells and HIV.* In the 1980s, several groups noted the presence of large numbers of HIV particles on the dendritic processes of FDCs within the germinal centers of secondary lymphoid organs of infected individuals (Armstrong and Horne, 1984; Biberfeld *et al.*, 1988; Fox and Cottler-Fox, 1992; Le Tourneau *et al.*, 1985; Tenner-Racz *et al.*, 1988). Such retention of C-type retroviruses had in fact already been recognized in the early days of FDC research (Hanna and Szakal, 1968; Hanna *et al.*, 1970; Szakal and Hanna, 1968). Virus trapping is most likely due to the formation of immune complexes that bind to Fc and complement receptors on FDCs. The HIV particles trapped within the FDC processes remain infectious for protracted periods of time (Heath *et al.*, 1995). The lymphadenopathy characteristic of the early stages of HIV infection is followed by the disappearance of the FDC network, which is in turn followed by follicle lysis and generalized immunosuppression (Fauci, 1996). Susceptibility to HIV replication cannot explain the disappearance of the FDC network because FDCs lack virus receptor and coreceptors and are therefore not permissive for HIV infection. The loss of FDCs may therefore be due to (1) the activation of CD8<sup>+</sup> CTLs that lyse FDCs in a bystander fashion or (2) the lack of T cell-dependent FDC growth and/or survival as a consequence of T cell exhaustion (Kapasi *et al.*, 1993). These issues are being addressed *in vivo* in the mouse MAIDS model induced by the murine leukemia retrovirus (Burton *et al.*, 1997).

*b. Dendritic Cells and HIV.* Because DCs express CD4, the receptor for HIV, early studies analyzed whether DCs would essentially act as (1) transporters of the virus, initially deposited on the mucosa, to activated T cells in secondary lymphoid organs (Cameron *et al.*, 1992) or (2) permissive sites for virus replication (Fauci, 1996; Langhoff *et al.*, 1991; Macatonia *et al.*, 1989, 1990; Weissman and Fauci, 1997; Weissman *et al.*, 1997). These studies eventually led to the finding that explosive HIV replication occurs when DCs and resting T cells are cocultured (Pinchuk *et al.*, 1994; Pope *et al.*, 1994, 1995). Although resting T cells, as opposed to activated T cells, are unable to support a productive infection, DCs can support low levels of virus replication consistent with their expression of multiple chemokine coreceptors (Aychunne *et al.*, 1997; Granelli-Piperno *et al.*, 1996; Zaitseva *et al.*, 1997). Infection and transmission may also vary with the maturational stage of DCs (Granelli-Piperno *et al.*, 1998). When immature and mature populations of DCs were generated from blood monocytes (using GM-CSF + IL-4 to provide DC<sub>imm</sub> cells, followed by

LPS to provide DC<sub>mat</sub>), the DC<sub>imm</sub> replicated M-tropic but not T-tropic HIV-1 whereas DC<sub>mat</sub> replicated both types of viruses but only in concert with T cells, and not as populations depleted of T cells.

Most of the viral production from these DC-T cell cocultures occurs within syncytia that are heterokaryons of DCs and T cells. Each cell type brings a specific transcription factor allowing viral genome expression. Specifically DCs provide high levels of active NF- $\kappa$ B whereas T cells provide the Sp1 transcription complex (Granelli-Piperno *et al.*, 1995). In accordance with these *in vitro* studies, HIV-expressing syncytia have been found *in vivo* at the surfaces of mucosal lymphoid tissues such as tonsils and adenoids (Frankel *et al.*, 1996, 1997).

Chemokine receptors, ordinarily considered most pertinent to immune cell trafficking and inflammation, have also proved critical to certain infectious disease processes. In the case of HIV, CR5 as well as CXCR4 can act as coreceptors for the virus (Wyatt and Sodroski, 1998). It has been demonstrated that HIV-1 can infect DCs *in vitro* through interactions with CCR5 and CXCR4 receptors (Ayehunie *et al.*, 1997; Rubbert *et al.*, 1998; Zaitseva *et al.*, 1997). Conflicting results revolve around the function of circulating DCs in AIDS patients. A deficit of circulating DCs observed early in infection (Macatonia *et al.*, 1989) may explain the early loss of CD4<sup>+</sup> memory T cells (Knight *et al.*, 1997), because the memory T cell pool *in vivo* has been shown to depend highly on the presence of functional DCs (Brocker *et al.*, 1997). It is hoped that an improved understanding of the pathogenic role of HIV in the DC system will facilitate the use of DCs to establish long-term immunity against HIV.

## H. DENDRITIC CELLS AND TUMORS

### I. General Considerations Regarding Tumor Immunity

The immune system has the potential to reject tumors as evidenced by occasional spontaneous remission of various types of cancer, e.g., renal cell carcinomas and melanomas (Boon *et al.*, 1994; Houghton, 1994). Tumor regression occurs when CTLs recognize class I MHC peptide complexes on the tumor cell surface. For this to occur, antigen-presenting cells (and more specifically DCs) should first home into the tumor, capture tumor antigens, then migrate to secondary lymphoid organs to initiate T lymphocyte responses against the tumor-associated antigens (TAAs).

Numerous studies over the past decade have now identified a large number of TAAs that can be categorized as (1) antigens encoded by genes that are completely silent in most normal tissues but activated in tumors (e.g., the MAGE, BAGE, GAGE genes that are expressed in most melanomas and many other tumors, but in normal tissue only in placenta and/or

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testis), (2) differentiation antigens present within a tumor cell as well as its normal counterpart [e.g., tyrosinase of melanocytes, or carcinoembryonic antigen (CEA)], (3) antigens resulting from mutations that are truly tumor-specific antigens that affect a wide variety of proteins, including those involved in cell growth (e.g., Her 2/neu, a growth factor receptor overexpressed in breast and ovarian carcinomas but present at low levels in some normal tissue), (4) overexpressed tumor antigens, and (5) viral antigens derived from oncogenic viruses [e.g., E7 oncoprotein of human papilloma virus (HPV) 16 found in most cervical carcinomas].

The final or efferent step of the antitumor immune response occurs when the primed TAA-specific CTLs leave the secondary lymphoid organs and return to the tumor to kill the malignant cells. Why then do cancers develop despite the immune system? What is the role of DCs in cancer development and/or regression?

### *2. Tumors with Increased Dendritic Cell Numbers Have a Better Prognosis*

Immunohistological analysis performed in the late 1980s and early 1990s using S100 staining as a marker for DCs demonstrated that an increased number of DCs located within tumors was associated with better prognosis. This has been described for colorectal adenocarcinoma (Ambe *et al.*, 1989), adenocarcinoma of the lung (Furukawa *et al.*, 1985; Fox, 1989), papillary carcinoma of the thyroid (Schroder *et al.*, 1988), as well as gastric (Tsujitani *et al.*, 1990), esophageal (Imai and Yamakawa, 1993), and nasopharyngeal (Nomori *et al.*, 1986) carcinomas.

### *3. Developing Tumors Contain Dendritic Cells with an Immature Phenotype*

Colon carcinomas display a heavy infiltrate of macrophages and/or DCs that express high levels of class II HLA antigens. However, these DCs marginally express CD80 and CD86 (Chaux *et al.*, 1996). Similar findings have been reported for basal cell carcinomas, wherein only 1–2% of intratumor and 5–10% of peritumor APCs expressed CD80 and CD86 (Nestle *et al.*, 1997) as well as reduced levels of CD40 (Viac *et al.*, 1997). Consistently, DCs isolated from basal cell carcinomas display low allostimulatory capacity as one indicator of altered immunogenicity. A recent detailed functional analysis of infiltrating DCs in responding versus progressing melanoma metastases in the same patient showed that DCs infiltrating the responding metastases have the characteristics of mature DCs, with potent allostimulatory properties and high levels of CD80, CD83, and CD86 (Enk *et al.*, 1997). In contrast, DCs within progressing melanoma metastases display reduced CD83 and almost no CD86, and they exert fivefold less

stimulation of allogeneic T cells than do DCs from the regressing metastases (Enk *et al.*, 1997). Importantly, *in vitro* assays measuring tolerance induction show that DCs from progressing metastases induce T cell anergy, whereas DCs from regressing metastases do not. The alteration of dendritic cell functions appears to go beyond the tumor site, because blood DCs from patients suffering from stage III and IV breast cancer show decreased allostimulatory capacity and decreased CD80-CD86 expression (Gabrilovich, 1997). DCs with altered functions have also been found in the spleens of tumor-bearing animals (Gabrilovich *et al.*, 1994).

Cancer cells also secrete factors that alter DC functions as well as development. Among these, IL-10 appears to play a critical role, as evidenced by IL-10 production by progressing melanoma metastases (Engering *et al.*, 1997) and by the absence of DC infiltration in experimental tumors secreting IL-10 *in vivo* (Qin *et al.*, 1997). Vascular endothelial cell growth factor (VEGF), which is produced by nearly all tumor cells, represents another candidate that affects the development of DCs from hematopoietic progenitors (Gabrilovich *et al.*, 1996). VEGF offer tumors the additional advantage of inducing endothelial cell growth and angiogenesis.

#### 4. Mature Dendritic Cells Presenting Tumor-Associated Antigen Can Cure Most Experimental Mouse Models of Cancer

Experiments over the past few years have demonstrated the feasibility of eradicating tumors in mice with DCs loaded with tumor-associated antigens. Initial studies, performed with tissue-derived DCs, concentrated on antitumor responses that were essentially MHC class II dependent (Cohen *et al.*, 1994; Flamand *et al.*, 1994; Grabbe *et al.*, 1991). However, potent MHC class I-restricted CD8 responses can also be induced *in vivo* by administration of Ag-pulsed DCs obtained from either tissues (Takahashi *et al.*, 1993) or cultured bone marrow cells (Porgador and Gilboa, 1995). DCs were initially loaded with Ag by pulsing defined peptides of known sequence (Celluzzi *et al.*, 1996; Mayordomo *et al.*, 1995) or undefined peptides isolated by acid elution from tumor cell lines (Zitvogel *et al.*, 1996). Genetically modified DCs have been shown to induce strong MHC-restricted CTL responses, resulting in considerable antitumor effects. Genetic modification has been performed either at the bone marrow precursor level using retroviral vectors (Specht *et al.*, 1997) or at the mature stage using replication-deficient, recombinant adenoviral vectors (Song *et al.*, 1997).

In most experimental models tested to date, the afferent sensitization arm of the response has required concomitant presentation of a xenogeneic peptide, e.g., OVA-peptide, in order for the elicited CTLs to recognize a

s from the regressing metastatic assays measuring tolerance. Do metastases induce T cell anergy, or not. The alteration of dendritic tumor site, because blood vessels and I and IV breast cancer show altered CD80-CD86 expression. These findings have also been found in ilovich *et al.*, 1994).

After DC functions as well as to play a critical role, as evidenced by melanoma metastases (Engelhard *et al.*, 1997). Vascular endothelial cells are induced by nearly all tumor cells, and the development of DCs from tumor cells (Zitvogel *et al.*, 1996). VEGF offers tumors a survival advantage by promoting tumor cell growth and angio-

#### Tumor-Associated Antigen Can Induce Antitumor Models of Cancer

It has been demonstrated the feasibility of using blood-derived DCs, concentrated by MHC class II dependent methods (Krabbe *et al.*, 1991). However, antitumor responses can also be induced *in vivo* by DCs obtained from either tissues or bone marrow cells (Porgador and Steinman *et al.*, 1995). Ag by pulsing defined peptides (Mayordomo *et al.*, 1995) derived from tumor cell lines (Zitvogel *et al.*, 1996) have been shown to induce antitumor responses in considerable antitumor models, either at the bone marrow (Specht *et al.*, 1997) or at the recombinant adenoviral vectors

site, the afferent sensitization and presentation of a xenogeneic antigen elicited CTLs to recognize a

parental tumor expressing only the tumor-specific antigen. For repeated vaccinations with DCs, the induction of antiviral or antivector immunity may also represent an important limiting step. In this regard, the successful loading of DCs with whole tumor cell-derived RNA (Boczkowski *et al.*, 1996) represents an interesting approach that would render feasible the presentation by DCs of both cytolytic and helper antigenic epitopes from small tumor samples. Autoantigens might also be simultaneously presented, however. Fusing DCs with tumor cells has also yielded antitumor responses in mice (Gong *et al.*, 1997; Gong, 1998), but this approach is difficult to implement in human therapy. Indeed, fusion per se may not be necessary, because physical contact between DCs and tumor cells may produce an immunogen that induces tumor protection and therapeutic tumor rejection (Celluzzi and Falo, 1998). Whether this results in the capture of tumor-derived apoptotic bodies remains to be determined. Interestingly, DC viability may not even be required for immunity to occur, as demonstrated by the ability of tumor peptide-pulsed DC-derived exosomes to prime specific CTLs *in vivo* and eradicate or suppress growth of established murine tumors in a T cell-dependent manner (Zitvogel *et al.*, 1998).

#### 5. Pilot Clinical Trials Indicate the Safety of Dendritic Cell Administration to Humans

Significant clinical responses have been observed in pilot trials using blood-derived dendritic cells loaded with lymphoma idiotype (Hsu *et al.*, 1996). Peptide-pulsed antigen-presenting cells generated by culturing monocytes with GM-CSF alone have also elicited *in vivo* immune responses (Mukherji *et al.*, 1995). Some clinical responses have also been observed in prostate cancer using DCs generated by culturing monocytes with GM-CSF + IL-4, then pulsed with prostate-specific membrane antigen peptide (Murphy *et al.*, 1996; Tjoa *et al.*, 1995, 1996, 1997). Melanoma peptide-pulsed DCs, also generated by culturing monocytes with GM-CSF + IL-4, induced clinical regression in 5 of 16 patients treated, two of the patients showing a complete response of all evaluable disease (Nestle *et al.*, 1998a). Longevity of the responses, as well as real variation of the observed responses from the natural history of the tumors or from the effects of other adjuvants used with DC immunizations, are outstanding unknowns.

Transposing to human cancer the encouraging results observed in mice after DC immunotherapy will require significant efforts for multiple reasons. First, cancer in humans is in no way comparable to the reproducible, well-defined, cell line-based animal models. Second, the complexity of the DC lineage, with diverse subsets, stages of maturation, and methods of generation, necessitates that each step be tested independently. Furthermore, the nature of the tumor antigens, and the optimal method for loading

DCs with those tumor antigens, represent additional parameters for careful analyses. Strategies that introduce antigen into DCs, but allow the DCs to select and tailor peptides for presentation on available MHC molecules, would circumvent the need to identify tumor-specific peptides with known HLA restrictions *a priori*. Such approaches would also offer the theoretical advantage of introducing both helper and cytolytic antigenic epitopes for the generation of effective CTLs. Route of administration, intravenous versus intracutaneous versus intranodal, the dose of DCs, and the frequency of injections also need to be established.

Assuming successful induction of strong antitumor CTL activity in patients after DC immunization, there are still caveats to the long-term success of DC-based immunotherapy of cancer. CTLs may not readily migrate to the tumor site. Tumor variants may lose the class I MHC expression required for CTL recognition (Jager *et al.*, 1997). Tumor variants may also lose expression of critical tumor antigens, or express surface molecules such as FasL (Walker *et al.*, 1997), or secrete cytokines such as IL-10 (Chen *et al.*, 1994) that inactivate CTLs. Patients may experience either tumor-related or drug-induced immune suppression that would render CTL priming inefficient *in vivo*, in which case CTL priming may best be accomplished *in vitro*, followed by adoptive transfer to the diseased host.

In spite of all these potential pitfalls, the prospects are bright for immunotherapy of human cancer and very probably other diseases, using *in vitro*-generated DCs. Accordingly, numerous investigators are embarking on studies in this arena. This level of scientific investigation should facilitate rapid answers to many important unknowns, especially whether *ex vivo* manipulation of DCs represents the "holy grail" of tumor immunology. An alternative approach may be to increase, directly, the levels of DCs *in vivo* that are capable of capturing tumor antigens and turning in specific immune responses. Accordingly, administration of Flt-3L to mice challenged with methylcholanthrene-induced fibrosarcoma has been shown to induce complete tumor regression in a significant proportion of mice and decreased tumor growth in the remaining mice (Lynch *et al.*, 1997). There is, however, some evidence that this effect may not be due to the generation of specific CTLs, but rather to the activation of NK cells by the Flt-3L-elicited DCs (L. Zitvogel, personal communication). The systemic administration of Flt-3L may also break tolerance to tumors based on a study showing that administration of Flt-3L to animals breaks tolerance induced by systemic administration of soluble ovalbumin (Pulendran *et al.*, 1998). The complexity of Flt-3L effects *in vivo*, however, is revealed by the enhanced induction of oral tolerance (Viney *et al.*, 1998), which can be observed for very low doses of Ag that are ineffective in controls. Such a tolerizing effect of Flt-3L has, however, not been reported for tumors. In

Additional parameters for careful selection of DCs, but allow the DCs to be selected on available MHC molecules, and for specific peptides with known epitopes would also offer the theoretical possibility of selecting cytolytic antigenic epitopes for administration, intravenous or intradermal dose of DCs, and the frequency

antitumor CTL activity in patients with cancer. CTLs may not readily kill tumor cells in vivo (Jäger *et al.*, 1997). Tumor cells may not express surface antigens, or express surface antigens that are not recognized by CTLs (Jäger *et al.*, 1997), or secrete cytokines such as TGF- $\beta$  that suppress CTLs. Patients may experience immunosuppression that would inhibit CTL activity. In which case CTL priming may be necessary, or by adoptive transfer to the dis-

prospect is bright for immunology and other diseases, using *in vitro* investigators are embarking on investigation should facilitate this, especially whether *ex vivo* "grail" of tumor immunology. Second, directly, the levels of DCs in antigens and turning in specific regulation of Flt-3L to mice chondrosarcoma has been shown to significant proportion of mice and mice (Lynch *et al.*, 1997). There may not be due to the generation of NK cells by the Flt-3L (Lynch *et al.*, 1997). The systemic administration to tumors based on a study in mice breaks tolerance induced by tumor (Pulendran *et al.*, 1998). However, it is revealed by the study (Lynch *et al.*, 1998), which can be ineffective in controls. Such a result has been reported for tumors. In

fact, in this context, DCs have been able to break tolerance to tumors that has been induced by tumor peptides administered with adjuvants (Toes *et al.*, 1996a,b, 1998).

## VIII. Concluding Remarks

It is now clear that DCs can no longer be considered the *parent pauvre* (poor relative, or black sheep) of the antigen-presenting cell family. DCs form a complex population of cells with the potential to engage in functions as contrasting as the induction of immunity versus the induction of tolerance. Much remains to be learned about these cells. In particular, the mechanisms regulating the balance between immunizing and tolerizing DCs must be investigated. The cellular and molecular events involved in T cell activation by DCs are becoming better established, but there are enormous deficits in the knowledge of how DCs could induce tolerance, especially in the periphery. Answers to these questions will permit the therapeutic manipulation of the DC system. Initially, defined DC populations generated *in vitro* will be administered to patients to induce either immunity (as required in cancer and infectious diseases) or tolerance (as required in allergy, autoimmunity, and transplantation). Finally, one may directly target DCs *in vivo* using specific pharmacologic agents. Although single agents such as steroids (Kitajima *et al.*, 1996) or Flt-3L exert effects on DCs in experimental models, more sophisticated strategies targeting various DC subpopulations and various stages of maturation will probably be necessary to enhance or inhibit specific immune responses with precise control. Although the tasks are immense, considerable means from academic, government, private, and industrial sources are now being devoted to DC research. It should not be long before DC-targeted therapy becomes part of numerous medical interventions.

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